THE
JOURNAL OF PHYSIOLOGY

EDITED BY

J. N. LANGLEY, Sc.D., F.R.S.

AIDED, IN THE SELECTION OF PAPERS FOR PUBLICATION, BY

Prof. W. M. BAYLISS (London).
Prof. T. G. BRODIE (Toronto).
Sir T. LAUDER BRUNTON, Bart. (London).
Sir DAVID FERRIER (London).
Dr J. S. HALDANE (Oxford).
Prof. W. D. HALLIBURTON (London).
Mr W. B. HARDY (Cambridge).
Mr F. G. HOPKINS (Cambridge).
Sir VICTOR HORSLEY (London).
Prof. A. B. MACALLUM (Toronto).
Prof. D. NOËL PATON (Glasgow).
Prof. W. A. OSBORNE (Melbourne).
Prof. C. S. SHERRINGTON (Oxford).
Prof. E. H. STARLING (London).
Prof. W. STIRLING (Manchester).
Prof. Sir ANDERSON STUART (Sydney).
Prof. A. WALLER (London).

Vol. XLVIII.

1914

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, Manager
LONDON: FETTER LANE, E.C.
EDINBURGH: 100, PRINCES STREET.

Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS.

CONTENTS OF VOL. XLVIII.

No. 1. *March* 31, 1914.

PAGE

Diuresis. By **Douglas Cow** 1

On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. By **T. Graham Brown** 18

Hyperpnœa as a result of pain and ether in man. By **A. L. Meyer** 47

The relation between the size of the propagated disturbance and the rate of conduction in nerve. By **E. D. Adrian** 53

The antagonism of curari and nicotine in skeletal muscle. By **J. N. Langley** 73

Nos. 2 & 3. *May* 18, 1914.

Further observations on the constricting action of adrenaline on the cerebral vessels. By **Carl J. Wiggers** 109

The influence of carbohydrate and fat on protein metabolism. III. The effect of sodium selenite. By **E. P. Cathcart** and **J. B. Orr** 113

The cerebro-spinal fluid. II. Cerebro-spinal pressure. By **W. E. Dixon** and **W. D. Halliburton** 128

Vaso-motor nerves in the lungs. By **Enid M. Tribe** . . . 154

On the effects of complete and incomplete castration upon horn growth in herdwick sheep. By **F. H. A. Marshall** and **J. Hammond** 171

The carbon dioxide production of heat rigor in muscle, and the theory of intra-molecular oxygen. By **W. M. Fletcher** and **G. M. Brown** 177

Observations upon decerebrate rigidity. By **Lewis H. Weed** . 205

Is polished rice plus vitamine a complete food? By **Casimir Funk** 228

No. 4. *July* 14, 1914.

The formation of urine in the frog. By **F. A. Bainbridge**, **J. A. Menzies**, and **S. H. Collins** 233

The absorption and dissociation of carbon dioxide by human blood. By **Johanne Christiansen**, **C. G. Douglas**, and **J. S. Haldane** . . 244

The influence of distention of the lungs on human respiration. By **Johanne Christiansen** and **J. S. Haldane** 272

PAGE

The heart, lung, kidney preparation. By F. A. BAINBRIDGE and C. L. EVANS 278

The ferments of the pancreas. Part IV. Steapsin. By JOHN MELLANBY and V. J. WOOLLEY 287

The sensitiveness of the respiratory centre to carbonic acid, and the dead space during hyperpnœa. By J. M. H. CAMPBELL, C. GORDON DOUGLAS and F. G. HOBSON 303

Cerebro-spinal fluid. III. The general effects of increasing the cerebro-spinal pressure. By W. E. DIXON and W. D. HALLIBURTON . 317

The influence of a vitamine-free diet on the carbohydrate metabolism. By CASIMIR FUNK and Count ERWIN v. SCHÖNBORN . . 328

On some cardiac reflexes. By F. A. BAINBRIDGE 332

The determination of lactic acid. By CHARLES G. L. WOLF. . 341

On the constancy of the systolic output under varying conditions. By JOSEPH MARKWALDER and E. H. STARLING. 348

No. 5. *September* 8, 1914.

On the mechanical factors which determine the output of the ventricles. By S. W. PATTERSON and E. H. STARLING 357

The time relations of acid production in muscle during contraction. By H. E. ROAF 380

On the origin of the "c" wave in the jugular pulse. By JAMES M. MCQUEEN and A. W. FALCONER 392

Myothermic experiments in salt-solutions in relation to the various stages of a muscular contraction. By VIKTOR WEIZSÄCKER . . . 396

Gaseous exchange in the decerebrate animal. By CHARLES G. L. WOLF and T. S. HELE 428

The suprarenal bodies and diuresis. By DOUGLAS COW . . . 443

The temperature coefficient of the refractory period in nerve. By E. D. ADRIAN 453

No. 6. *October* 23, 1914.

The regulation of the heart beat. By S. W. PATTERSON, H. PIPER, and E. H. STARLING 465

LIST OF AUTHORS.

ADRIAN, E. D. Temperature Coefficient 453

ADRIAN, E. D. Conduction in Nerve 53

BAINBRIDGE, F. A., MENZIES, J. A., and COLLINS, S. H. Formation of Urine 233

BAINBRIDGE, F. A. Cardiac Reflexes 332

BAINBRIDGE, F. A., and EVANS, C. L. Heart, Lung, Kidney Preparation 278

BROWN, T. G. Fundamental Nervous Activity 18

PAGE

Brown, G. M., and Fletcher, W. M. Heat-Rigor and Intra-Molecular Oxygen . . . 177

Campbell, J. M. H., Douglas, C. G., and Hobson, F. G. Respiratory Centre and Dead Space . . . 303

Cathcart, E. P., and Orr, J. B. Selenium on Metabolism . . . 113

Christiansen, J., and Haldane, J. S. Distention of the Lungs . . . 272

Christiansen, J., Douglas, C. G., and Haldane, J. S. Carbon Dioxide in Blood . . . 244

Collins, S. H., Bainbridge, F. A., and Menzies, J. A. Formation of Urine . . . 233

Cow, D. Suprarenals and Diuresis . . . 443

Cow, D. Diuresis . . . 1

Dixon, W. E., and Halliburton, W. D. Cerebro-Spinal Fluid . . . 317

Dixon, W. E., and Halliburton, W. D. Cerebro-Spinal Fluid . . . 128

Douglas, C. G., Christiansen, J., and Haldane, J. S. Carbon Dioxide in Blood . . . 244

Douglas, C. G., Campbell, J. M. H., and Hobson, F. G. Respiratory Centre and Dead Space . . . 303

Evans, C. L., and Bainbridge, F. A. Heart, Lung, Kidney Preparation 278

Falconer, A. W., and McQueen, J. M. "C" Wave in Jugular . . . 392

Fletcher, W. M., and Brown, G. M. Heat-Rigor and Intra-molecular Oxygen . . . 177

Funk, C. Rice and Vitamine . . . 228

Funk, C., and Schönborn, Count E. v. Vitamine-Free Diet . . . 328

Haldane, J. S., and Christiansen, J. Distention of the Lungs . . . 272

Haldane, J. S., Christiansen, J., and Douglas, C. G. Carbon Dioxide in Blood . . . 244

Halliburton, W. D., and Dixon, W. E. Cerebro-Spinal Fluid . . . 128

Halliburton, W. D., and Dixon, W. E. Cerebro-Spinal Fluid . . . 317

Hammond, J., and Marshall, F. H. A. Castration and Horn Growth 171

Hele, T. S., and Wolf, C. G. L. Gaseous Exchange . . . 428

Hobson, F. G., Campbell, J. M. H., and Douglas, C. G. Respiratory Centre and Dead Space . . . 303

Langley, J. N. Antagonism of Poisons . . . 73

Markwalder, J., and Starling, E. H. Systolic Output . . . 348

Marshall, F. H. A., and Hammond, J. Castration and Horn Growth 171

McQueen, J. M., and Falconer, A. W. "C" Wave in Jugular . . . 392

Mellanby, J., and Woolley, V. J. Steapsin . . . 287

Menzies, J. A., Bainbridge, F. A., and Collins, S. H. Formation of Urine . . . 233

Meyer, A. L. Hyperpnœa in Man . . . 47

Orr, J. B., and Cathcart, E. P. Selenium on Metabolism . . . 113

Patterson, S. W., and Starling, E. H. Output of Ventricles . . . 357

Patterson, S. W., Piper, H., and Starling, E. H. Heart Beat Regulation . . . 465

PAGE
Piper, H., Patterson, S. W., and Starling, E. H. Heart Beat Regulation 465
Roaf, H. E. Acid Production and Contraction 380
Schönborn, Count E. v., and Funk, C. Vitamine-Free Diet 328
Starling, E. H., and Markwalder, J. Systolic Output 348
Starling, E. H., and S. W. Patterson. Output of Ventricles 357
Starling, E. H, Patterson, S. W., and Piper, H. Heart Beat Regulation 465
Tribe, E. M. Vaso-motor Nerves of Lungs 154
Weed, L. H. Decerebrate Rigidity 205
Weizsäcker, V. Myothermic Experiments 396
Wiggers, C. J. Adrenaline on Cerebral Vessels 109
Wolf, C. G. L. Lactic Acid Estimation 341
Wolf, C. G. L., and Hele, T. S. Gaseous Exchange 428
Woolley, V. J., and Mellanby, J. Steapsin 287

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY.

January 24, 1914.

Burridge, W. Note on the hypodynamic heart i
Homer, Annie. A note on the excretion of kynurenic and uric acids by dogs ii
Dale, H. H. The occurrence in ergot and action of acetyl-choline. (*Preliminary communication*) iii
Homer, Annie. A note on a new method of estimating tryptophane in proteins iv
Edridge-Green, F. W. The homonymous induction of colour v
Mines, G. R. The action of muscarine on the electrical response of the frog's heart. (*Preliminary note*) vi
Peters, R. A. A combined tonometer and electrode cell for measuring the H ion concentration of reduced blood at a given tension of CO_2. (*Preliminary communication*) vii

February 14, 1914.

Burridge, W. The influence of sodium chloride on the frog's heart. (*Preliminary communication*) ix
Hill, A. V. The oxidative removal of lactic acid x
Hill, A. V. The total energy available in isolated muscles kept in oxygen xi
Hill, A. V. and Hill, A. M. A self-recording calorimeter for large animals xiii
Martin, C. J. A simple and convenient form of bicycle ergometer xv
Waller, A. D. The electrical axis of the heart in systole and in diastole xvii

March 14, 1914.

Burridge, W. Some effects of barium chloride. (*Preliminary communication*) xix
Mellanby, J. and Woolley, V. J. The preparation of pancreatic extracts xx
Cathcart, E. P. and Orr, J. B. The influence of acetoacetic acid on the estimation of creatinine xxi

PAGE
Kent, Stanley A. F. The right lateral auriculo-ventricular junction of the heart xxii
Dale, H. H. and Ewins, A. J. Choline-esters and muscarine. (*Preliminary communication*) xxiv
Buckmaster, George A. The action of hydrazine hydrate on methæmoglobin xxv
Edridge-Green, F. W. and Porter, A. W. Demonstration of the negative after-images of spectral and compound colours of known composition xxvi
Mac William, J. A., Melvin, G. Spencer, and Murray, J. R. Blood-pressure estimation by the auditory method xxvii

May 16, 1914.

Brown, T. Graham. The phenomenon of augmentation of excitability in the motor cortex xxix
Brown, T. Graham. Motor activation of the post-central gyrus . . xxx
Brown, T. Graham. A respiratory tract in the mid-brain xxxii
Brown, T. Graham. Note on the functions of the post-central gyrus in the anthropoid ape xxxiii
Macdonald, J. S. Mechanical efficiency of man xxxiii

June 6, 1914.

Hill, A. V. and Weizsäcker, Vicktor. Improved myothermic apparatus . xxxv
Weizsäcker, Vicktor. The effects of various physical and chemical factors on the initial heat-production of muscle xxxvi
Edridge-Green, F. W. Binocular vision xxxviii
Burridge, W. Some points in the action of adrenin xxxix
Waller, A. D. Voluntary reversal of the human electrocardiogram by deep respiration xl
Krause, R. A. The effect of acetone and aceto-acetic acid on the estimation of creatinine and creatine xli
Krause, R. A. The excretion of creatine in severe and mild cases of diabetes mellitus xliii
Lindhard, J. The "dead space" in breathing xliv
Waller, Mary D. Note on the relation between the electrolyte concentration of some neutral perfusion liquids and the frequency of beat of the frog's heart xlvi
Waller, W. W. Excitation of the sudo-motor nerves of the cat's foot by condenser discharges xlviii

June 27, 1914.

Hartridge, H. and Hill, A. V The infra-red absorption bands of hæmoglobin li
Graham, G. and Poulton, E. P. Creatinine and creatine in starvation . liii
Graham, G. and Poulton, E. P. The removal of aceto-acetic acid from urine in creatinine and creatine determinations lv
Lamb, F. W. The oxidation of cholesterol lvi
Kent, Stanley A. F. A conducting path between the right auricle and the external wall of the right ventricle in the heart of the mammal . lvii
Burridge, W. Some actions of magnesium chloride lviii
Burridge, W. Note on the potassium contractions lviii
Burridge, W. Some effects of epinine lx
Burridge, W. Adrenin and perfusing solutions lxi
Burridge, W. Equilibrium in salt perfusing solutions lxi
Kent, Stanley A. F. Illustrations of the right lateral auriculo-ventricular junction in the heart lxiii

LIST OF AUTHORS.

		PAGE
Brown, T. Graham.	Excitability of motor cortex	xxix
Brown, T. Graham.	Activation of post-central gyrus	xxx
Brown, T. Graham.	Respiratory tract	xxxii
Brown, T. Graham.	Functions of post-central gyrus	xxxiii
Buckmaster, G. A.	Methæmoglobin	xxv
Burridge, W.	Magnesium on heart	lviii
Burridge, W.	Potassium on heart	lviii
Burridge, W.	Epinine on heart	lx
Burridge, W.	Adrenin	lxi
Burridge, W.	Salt in perfusing fluids	lxi
Burridge, W.	Hypodynamic heart	i
Burridge, W.	Sodium chloride on heart	ix
Burridge, W.	Barium chloride on heart	xix
Burridge, W.	Adrenin	xxxix
Cathcart, E. P. and Orr, J. B.	Estimation of creatinine	xxi
Dale, H. H.	Acetyl-choline	iii
Dale, H. H. and Ewins, A. J.	Choline-esters	xxiv
Edridge-Green, F. W.	Colour induction	v
Edridge-Green, F. W.	Binocular vision	xxxviii
Edridge-Green, F. W. and Porter, A. W.	After-images	xxvi
Graham, G. and Poulton, E. P.	Creatine in starvation	liii
Graham, G. and Poulton, E. P.	Creatine in starvation	lv
Hartridge, H. and Hill, A. V.	Spectrum of hæmoglobin	li
Hill, A. V. and Hill, A. M.	Recording calorimeter	xiii
Hill, A. V.	Available energy in muscle	xi
Hill, A. V.	Lactic acid	x
Hill, A. V. and Weizsäcker, Viktor.	Myothermic apparatus	xxxv
Homer, Annie.	Estimation of tryptophane	iv
Homer, Annie.	Kynurenic acid	ii
Kent, A. F. Stanley.	A.-V. junction	xxii
Kent, A. F. Stanley.	Conduction in A.-V. junction	lvii
Kent, A. F. Stanley.	A.-V. junction	lxiii
Krause, R. A.	Estimation of creatinine	xli
Krause, R. A.	Creatine in diabetes	xliii
Lamb, F. W.	Cholesterol	lvi
Lindhard, J.	Dead space	xliv
Macdonald, J. S.	Efficiency of man	xxxiii
MacWilliam, J. A., Melvin, G. Spencer and Murray, J. R.	Blood-pressure	xxvii
Martin, C. J.	Bicycle ergometer	xv
Mellanby, J. and Woolley, V. J.	Pancreatic extracts	xx
Mines, G. R.	Muscarine on heart	vi
Peters, R. A.	H ion apparatus	vii
Waller, A. D.	Axis of heart	xvii
Waller, A. D.	Respiration on electrocardiogram	xl
Waller, M. D.	Electrolytes on heart	xlvi
Waller, W. W.	Sudo-motor nerves	xlviii
Weizsäcker, Viktor.	Heat production in muscle	xxxvi

DIURESIS. BY DOUGLAS COW,

Beit Memorial Research Fellow.

(*From the Pharmacological Laboratory, Cambridge.*)

THERE is a certain discrepancy between the results recorded by different observers of the diuretic effect of water and of saline solutions. The majority of observers have injected saline solutions intravenously and have noted a consequent increase in diuresis. Thus Bock and Hoffmann(2), Limbeck(9), Sobieranski(11), Dastre and Loye(4), Hamburger(7), Heidenhain(8), Magnus(10), Thompson(12), Barcroft and Straub(1) and others have recorded a diuretic effect as the result of injections of saline solutions, either hypotonic, isotonic or hypertonic.

Frey(5) on the other hand finds that intravenous injections of water and of hypotonic saline solutions do not produce an increase in diuresis. Ginsberg(6), too, experimenting on unanæsthetized dogs, in which permanent bladder cannulæ had been fitted, finds that varying amounts of water given by the mouth produce an increase in diuresis which is approximately proportional to the amount ingested, whilst the same and larger quantities injected subcutaneously or intravenously produce no corresponding increase in diuresis, though similar injections of 1·5 °/₀ solutions of sodium sulphate (Glauber's salt) are distinctly diuretic.

There is agreement, then, that water given by the mouth produces an increase in diuresis; but the result of subcutaneous or intravenous injections is not so certain.

In a previous paper(3) I have recorded the results of certain experiments bearing on this subject. Repeating Ginsberg's experiments I obtained very similar results, viz. that water given by the mouth produces an increase in diuresis, whilst water given subcutaneously or intravenously produces no such effect. Further I found that a watery extract of the stomach and duodenum of a freshly killed dog, when injected subcutaneously into another dog, produced an increase in diuresis which was exactly comparable to that produced by administering a similar quantity of water by the mouth. In all these experiments the excretion of urine was measured by withdrawing the urine from the bladder every 10 minutes and weighing the fluid so withdrawn. These experiments were repeated, but were carried out on two animals only.

In continuing this work I have employed two methods:—(1) using unanæsthetized dogs, and (2) using cats anæsthetized with urethane.

(1) *Observations on unanæsthetized dogs.* The method was similar to that employed in my earlier experiments, that is using unanæsthetized dogs and weighing the 10-minute output of urine; the only difference between the experiments described in my earlier paper on this subject and these was that in the former permanent bladder-cannulæ fitted with stop-cocks were used, and in the latter a soft rubber (Jacques') urethral catheter was kept *in situ* during the experiments which were carried out on female dogs.

As a result of 75 observations I am satisfied that it is possible to obtain quite as reliable readings of the volumetric excretion of urine by means of the catheter as by the more severe method of implanting a permanent metal cannula in the bladder. The detail of my procedure is a follows: the soft rubber catheter is introduced into the urethra by means of a glass speculum, the urine is drawn off from the bladder at the commencement of each experiment, and during the experiments the urine drawn off is collected every 10 minutes and weighed. During the last minute of each 10-minute period the catheter is gently manipulated. I consider this manipulation essential if even results are to be obtained by means of the catheter, otherwise the amount of residual urine in the bladder is apt to vary from one period to another. The only disadvantage of this method appears to be that the animals have to be kept fastened in slings during the experiments, whereas those with the permanent bladder-cannulæ are free to move about.

As a result of 65 observations on three dogs (in addition to those observations recorded in my earlier paper) I am convinced that success in this method is largely a matter of chance, and depends on the nature of the animal used, Though a considerable time was given to training these animals and to accustoming them to the retention of the catheter, to the passage of the stomach-tube and to the other manipulations that are necessary, only one of the three animals gave really satisfactory and reliable results. Thus one dog always panted profusely after subcutaneous injections of extracts, so that whilst an increase in diuresis could always be observed after water had been given by the mouth, no positive result was ever obtained following an injection. Nor was this surprising when one considered the amount of water given off by the mouth both in the form of aqueous vapour and in the form of actual drops of fluid (saliva). No accurate estimation of the amount of fluid lost by the organism in this way was obtained, but on several occasions a rough estimate of the amount of fluid lost by the mouth in the form of saliva was made (no account being taken of the probably larger quantity lost in the form of water-vapour). On three such occasions 8·3 c.c., about 10 c.c. and 6 c.c. were estimated in this way as being lost in 70, 70 and 40 minutes respectively. On each occasion an injection of an extract of gastro-intestinal mucous membrane had been given, and on each occasion the panting was greatest at the time when the increase in diuresis would have been expected, viz. about 70–80 minutes after the injection.

Another animal, again, was very susceptible to nervous influences: rarely could any increase in diuresis be obtained even as a result of giving water by the mouth, except in very large amounts, and when an increase did manifest itself it was frequently small in amount and often abruptly curtailed, as the result apparently of extraneous causes, such as the sound of a person walking in an adjoining room. Only by giving very large amounts of water, such as 300 c.c. or more, could any increase in diuresis be obtained that was at all comparable to the amount of fluid ingested; and this was too large an amount to use for injections.

Ginsberg (*loc. cit.*) notes this same variability as between different animals, attributing the difference in reaction to variations in the amount of food the animals had taken previously. Whilst there is no doubt that an animal which has been deprived of food will excrete less urine as a result of a given volume of water ingested than an animal which is sufficiently well fed (and this quite apart from the diuresis resulting from the actual digestion of food), there are other factors concerned. The five animals, on which my observations were carried out, were kept on fixed diets; a weighed daily meal, in four cases of fresh meat with or without a measured quantity of water, and in the other case of Melox with a measured amount of water, being served to each animal at 6 p.m. every day. No difference was noticeable between the reactions of the meat-fed and the Melox-fed animals.

Whilst the same animal, then, will generally react in the same way, there may be wide differences between the reactions given by any two animals: in fact for the purpose of this work many animals, however well-trained, may be quite useless when this method of observation is employed, as the diuretic effect of a substance may be masked by other events, particularly loss of water by the lungs and by reflexes of an inhibitory nature. It is of course perfectly well recognized that the latter influence may play an important part in the excretion of urine in man.

Owing to this cause I was led to two erroneous conclusions in my previous paper, in which I stated that injections of an extract of stomach and duodenum (1) previously boiled and (2) previously heated to 60° C. for 30 minutes were without diuretic effect. As a result of further investigation I wish to rectify these conclusions and to state that neither boiling nor heating to 60° C. destroys the diuretic property of gastro-intestinal extracts.

With this exception my more recent observations on unanæsthetized dogs have confirmed my previous results, and amplified them as follows:

(i) A diuretic substance can be extracted from the mucous membrane of the stomach and duodenum.

(ii) The mucous membrane of the jejunum also, and to a smaller extent that of the ileum, affords a substance which can be extracted, and which on injection produces an increase in diuresis.

On no occasion was any appreciable increase in body temperature noted by the rectal thermometer in the animals after injection.

(2) *Observations on cats anæsthetized with urethane.* The animals were as large as could be obtained, and were anæsthetized first with A.C.E. mixture and afterwards with urethane (1 gr. per kilo. of body-weight). The usual procedure was as follows:—as soon as the animal was anæsthetized an artificial respiration tube was tied into the trachea, so as to be in readiness in case of emergency: a glass cannula was inserted in the carotid artery and connected with a mercurial manometer for the blood-pressure record: a glass cannula was inserted in either the jugular or the femoral vein for purposes of injecting: on some occasions a cannula was inserted in the splenic tributary of the portal vein in addition, in order to make the injections directly into the portal circulation. In some cases the animals were eviscerated (*vide* Barcroft and Straub, *loc. cit.*): in other cases the vagi were severed in the neck. Unless evisceration or section of the vagi was performed the blood-pressure after a time usually sank so low that all diuresis came to a standstill. If one or the other (or both) of these precautions were taken, and if the dose of urethane did not exceed 1 gr. per kilo. of body-weight, the blood-pressure usually remained fairly constant at a height of about 80–90 mm. Hg. for five, six or even more hours after the commencement of the experiments.

After repeated trials of various kinds of bladder-cannulæ, I have used a metal cannula which I devised. This consists essentially of a hollow metal sphere of about 15 mm. diameter, through which as many small holes as possible are drilled, and into which a metal tube of about 2 mm. bore is brazed in such a way that one end projects to the centre of the metal sphere, whilst the part of the tube external to the sphere is about 30 mm. in length and is provided with a collar in order to prevent the cannula from slipping beneath the ligature (Fig. 1).

The method which I have adopted as routine for the insertion of the "cage-cannula" is as follows:—the urethra is ligatured close to the neck of the bladder, in order to prevent the bladder from contracting and emptying itself whilst being handled: an incision is made in the fundus of the bladder and the cannula introduced, the cut edges of the bladder being ligatured round the collar of the cannula. A rubber tube is connected to the distal end of the cannula and is passed through a hole pierced in the linea alba about midway between the sternum and the symphysis pubis. In this way the bladder is held in its normal position in the body: no kinking of the ureters is possible, and the special form of the cannula effectually prevents any alteration in the capacity of the bladder from time to time, since the bladder-wall remains in contact with the spherical part of the cannula, whilst the numerous holes which pierce it allow free passage to the urine as it escapes from the ureters. On no occasion was there found to be any distention of the ureters due to occlusion of their orifices from pressure on the spherical part of the cannula. There was a negative pressure of about 5 cms. of water throughout in the outflow tube. The only difficulty which has presented itself in using this cannula is the occasional presence of a bubble of air within the cannula or the tube leading away from it. This, however, is easily overcome by the expedient of adjusting the rubber tube to the cannula and filling both with warm saline before introducing the cannula into the bladder. The urinary flow was measured by a drop recorder.

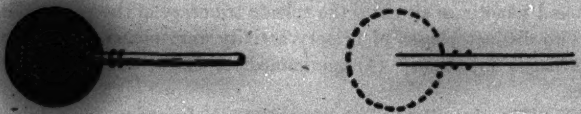


Fig. 1.

The animals were kept on a warm stage throughout the experiments, which were often continued for six hours or longer. About half an hour was always allowed to elapse after the completion of all manipulative proceedings, in order that the blood-pressure and flow of urine might become steady before commencing the experiments. By taking this precaution it was almost invariably found that the experiments were commenced with the flow of urine gradually diminishing: indeed on a few occasions on which the animals were left untouched and no injections given for a longer period, the decline in the rate of excretion of urine was progressive and constant. A record of such a decline, taken over a period of rather more than three hours, gives the following figures:

at 11.15, 40 drops of urine in 10 minutes
11.45, 34 " " " "
12.15, 28 " " " "
12.45, 16 " " " "
1.15, 12 " " " " and so on.

Injections were made intravenously in all cases, as a rule through a cannula introduced into the jugular vein, sometimes into the femoral vein and sometimes also into the splenic tributary of the portal vein.

Two distinct phases of urinary activity were observed as following intravenous injections: (1) an immediate effect, and (2) a delayed effect.

(1) *The immediate effect* was noticed as following every injection, whether of saline solution or of extracts of gastro-intestinal mucous membrane or of other substances. Immediately following the injection came a latent period of about 30 seconds, during which the flow of urine was perceptibly diminished: this was followed by a rapid increase in the flow of urine which reached its height about one minute later (*i.e.* about 1½ minutes after the injection), and then gradually diminished till the former rate of flow was reached, usually about 4–6 minutes after the injection. After this the flow of urine remained fairly constant at or about its original level for a considerable time.

Though this immediate effect was produced by injections both of saline solution and of extracts of gastro-intestinal mucous membrane, it was always far more pronounced after the injection of an active extract than after an injection of the same amount of saline. Thus in a typical experiment an injection of 10 c.c. of Ringer's solution was followed by a 40 % increase in the flow of urine, whilst an injection of 10 c.c. of an extract of duodenal mucous membrane in Ringer's solution provoked an increase of 200 % in the output of urine (Fig. 2).

Succeeding doses of the same extract produced less and less effect, thus on one occasion 5 c.c. of an extract of muscle in Ringer's solution (used as a control) produced a 10 °/。 increase in the flow of urine; following this 5 c.c. of an extract of the mucous membrane of the stomach and duodenum in Ringer's solution produced an increase of 165 °/。; three further injections of the same amount of this extract produced increases of 40 °/。, 26 °/。 and 0 °/。 respectively in the flow of urine (Fig. 3).

On one or two occasions an injection of Ringer's solution following shortly after an injection of an active extract was followed by an increase in the flow of urine as large as or even larger than that caused by the injection of the extract (Fig. 4); though, as was mentioned above, an initial injection of Ringer's solution never produced so large an increase in the flow of urine as that produced by the injection of an active extract.

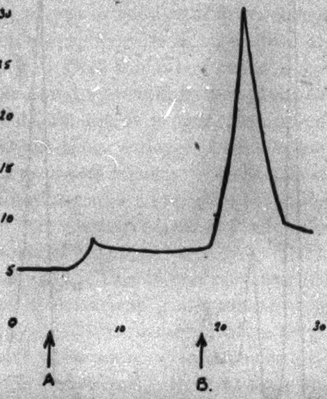


Fig. 2. Ordinate=drops of urine per 10 minutes. Abscissa=time in minutes. At *A*, 10 c.c. Ringer's solution given intravenously; at *B*, 10 c.c. extract of duodenal mucous membrane intravenously.

When successive injections of Ringer's solution were given, the resulting increase in diuresis became progressively larger after each injection: thus the first injection might produce an increase from 10 to 15 drops, the second from 12 to 20 drops, the third from 14 to 30 drops, and so on.

The greatest increases in the flow of urine were obtained by injections of extracts of duodenal mucous membrane. Extracts of mucous membrane of the stomach, jejunum and ileum also produced an increase, whilst extracts of no other substances that were used (including muscle, mucous membrane of the large intestine, mesentery and omentum, liver and fæces) produced any more effect than an initial injection of Ringer's solution, with the exception of an extract of pancreas, which sometimes

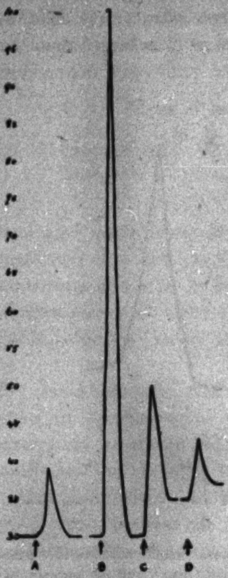


Fig. 3.

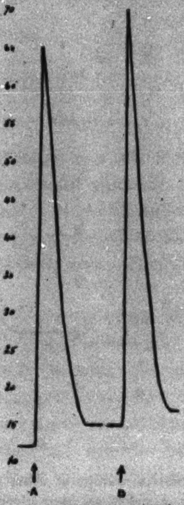


Fig. 4.

Fig. 3. Ordinate = drops of urine per 10 minutes. Abscissa = time ($\frac{1}{10}$ inch = 1 minute). At *A*, 5 c.c. extract of muscle intravenously; at *B*, *C* and *D*, 5 c.c. extract of gastric and duodenal mucous membrane intravenously.

Fig. 4. Ordinate = drops of urine per 10 minutes. Abscissa = time ($\frac{1}{10}$ inch = 1 minute). At *A*, 10 c.c. extract of gastric and duodenal mucous membrane intravenously; at *B*, 10 c.c. Ringer's solution intravenously.

produced a slight increase, not to be compared with that produced by extracts of gastro-intestinal mucous membrane.

No appreciable difference could be determined between the effects produced by injecting directly into the portal circulation and into the general circulation.

No definite relationship could be established between the blood-pressure and the immediate effect on the outflow of urine: thus after an injection of Ringer's solution the blood-pressure rose rapidly and returned more gradually to its previous level whilst the flow of urine was increasing. After an injection of an extract of gastro-intestinal mucous membrane on the other hand the blood-pressure fell rapidly and then rose again more gradually whilst the flow of urine was increasing.

When the kidney-volume was taken by an oncometer it was found that it increased after injections both of Ringer's solution and of gastro-intestinal extracts.

This immediate increase in the flow of urine, though sharply defined when measured by the drop-recorder, is short-lived and of little consequence in so far as the total amount of urine is concerned: indeed when the urine is measured by weighing the output at regular intervals it is hardly noticeable, though it is true that, when this method of measuring the urine has been employed, I have remarked the fact that the first measurement after an injection (*i.e.* the amount of urine voided during the first ten minutes after an injection) often does slightly exceed the amounts voided immediately before and after, though this excess is negligible as compared with

(2) *The delayed effect.* This is produced by: (*a*) giving water by the mouth, (*b*) injecting extracts of gastro-intestinal mucous membrane either subcutaneously or intravenously; but *not* by subcutaneous or intravenous injections of water or of saline when given in the physiological amounts which I have used. In these experiments I have never given more than the amount of fluid which the animal might be expected to take voluntarily in the physiological state: thus in experiments on anæsthetized cats of about 2·5–3 kilos. I have as a rule confined my observations to injections up to 12 c.c. in amount, and in the case of dogs of about 15 kilos. I have rarely exceeded 50 c.c. It seems that the unphysiological amounts of fluid which have been administered, usually intravenously, by other observers, notably by Barcroft and Straub, Dastre and Loye and Bock and Hoffmann, produce quite different results.

These experiments on anæsthetized cats have amply confirmed my previous results obtained with unanæsthetized dogs, viz. that, whilst a given volume of water by the mouth produces an increase in diuresis with its height occurring about 70 minutes after ingestion, similar amounts of water or of saline injected subcutaneously or intravenously produce no such increase in diuresis. (As in my earlier paper on this subject, I have on one or two occasions recorded an increase following injections of saline, but these increases have always been so small in amount as not to be compared with those produced by giving water by the mouth or by giving injections of extracts of gastro-intestinal mucous membrane. Moreover it is in my experience the exception for an injection of water or of saline to be followed by an increase in diuresis.) Also, as in my previous paper, I have found injections of extracts of gastro-intestinal mucous membrane to produce an increase in diuresis quite comparable to that produced by giving water by the mouth.

The extracts of gastro-intestinal mucous membrane (usually prepared from the mucous membrane of the duodenum, as this has been found to give the most active extracts) have been made as follows:—the mucous membrane of the duodenum of a freshly killed animal is scraped off and ground up with sand in either water or normal saline solution (*a*) at laboratory temperature, (*b*) boiling, (*c*) at laboratory temperature and acidulated with HCl, (*d*) boiling and acidulated with HCl. When acid is used the extract is afterwards made faintly alkaline with $NaHCO_3$. The extracts are filtered repeatedly and preserved in an ice-chest with either toluol or chloretone. The majority of the experiments were performed with freshly prepared extracts.

It was found that those extracts made in acidulated saline at laboratory temperature were as a rule the most active, though those prepared in normal saline at laboratory temperature and in boiling saline whether acidulated or not were also active, though not to the same extent as those extracts made in acidulated saline at laboratory temperature. Subsequent boiling does not appear to render these extracts inactive (Fig. 5).

Different extracts made in the same way appear to vary slightly in their activity, though the various extracts were made up as far as possible of the same strength: thus the usual procedure was as follows: a piece of duodenum six inches long was taken and the mucous membrane scraped from this, the amount of saline added being 30 c.c. This was further diluted before injection, one part of the extract to three parts of saline being the usual strength employed. Similarly with extracts of other substances, an approximately equivalent amount of the substance being extracted in the same quantity of saline.

These extracts appear to lose their activity somewhat on keeping; thus some extracts became almost inactive after keeping for four, five or six days, whilst on other occasions extracts that had been kept 10 days or longer appeared to have retained their activity unimpaired.

A typical experiment gives the following results:

at 12.30, 12 c.c. Ringer's solution injected into jugular;
12.20–2.0, 4·5 c.c. urine excreted;
2.0, 12 c.c. extract of gastric and duodenal mucous membrane in Ringer's solution injected into jugular;
2.0–3.30, 8 c.c. urine excreted. (In the following two hours a further 9 c.c. was excreted.) (Fig. 6.)

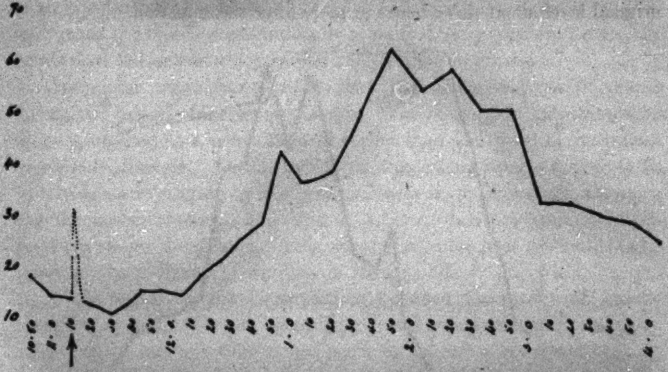


Fig. 5. Ordinate=drops of urine per 10 minutes. Abscissa=time in hours and minutes. At the mark indicated, 12 c.c. extract of duodenal mucous membrane, boiled before injection, intravenously. The dotted portion of the curve indicates the "immediate increase."

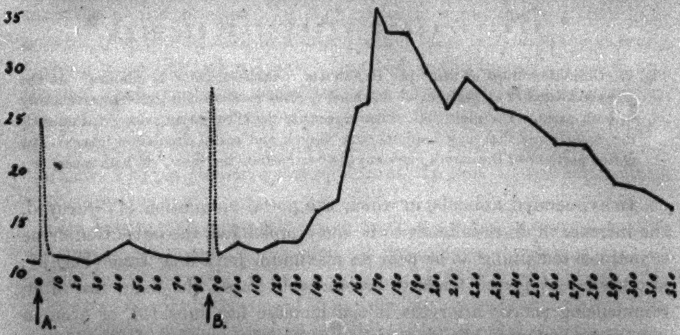


Fig. 6. Ordinate=drops of urine per 10 minutes. Abscissa=time in minutes. At *A*, 12 c.c. Ringer's solution intravenously; at *B*, 12 c.c. extract of duodenal mucous membrane in Ringer's solution. The dotted portions of the curve represent the "immediate increases" produced by the two injections.

As mentioned above some of these experiments were performed on eviscerated animals. In these there was as a rule a slight difference in the type of the diuresis resulting from the injection of extracts. In the animal with its viscera and portal circulation intact the output of urine, after the initial increase has subsided, remains as a rule at or about its normal level for perhaps 20–30 minutes after the injection, then rather rapidly increases, reaching its maximum about 70 minutes after the injection, after which the output of urine gradually declines, reaching its original level about three hours or more after the injection.

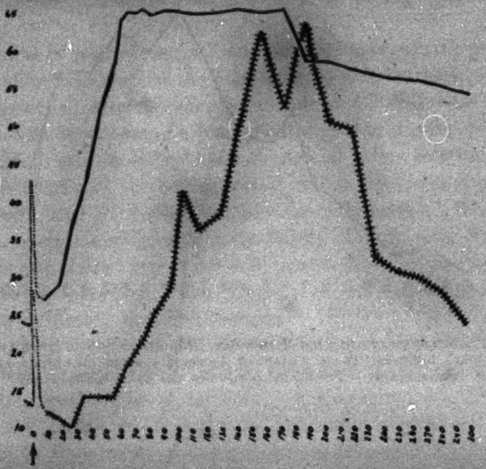


Fig. 7. Ordinate = drops of urine per 10 minutes. Abscissa = time in minutes. At the point indicated 12 c.c. extract of duodenal mucous membrane injected intravenously in both cases. The plain line curve represents the effect on an eviscerated animal, the crossed line that on an animal with viscera and portal circulation intact. The dotted portions of the curves represent the "immediate increases" in both cases.

In eviscerated animals, in which the portal circulation is destroyed, the increase in diuresis tends to be more prolonged, the output of urine sometimes remaining at or near its maximum for a long time (Fig. 7): also the latent period tends to become obliterated, the main rise often commencing shortly after the initial increase has subsided, or even in one or two instances blending with this initial rise. It is interesting to compare with this the result of Sterns(13) "occlusion of the liver is followed by a cessation of diuresis."

As a rule this increase in diuresis lasts longer in the anæsthetized cat than in the unanæsthetized dog; presumably less fluid is lost by the respiration in the former than the latter; the unanæsthetized dog may pant, whilst in the cat under the influence of urethane respiration is as a rule shallow.

No definite relationship could be established between the blood-pressure and the output of urine. The initial increase in diuresis always followed a sharp fall in blood-pressure (vaso-motor in type) in the case of an injection of an extract. After this and during the delayed increase in diuresis the blood-pressure usually remained fairly constant: certainly no marked fall or rise accompanied the increase in diuresis.

Oncometer experiments show that the kidney-volume increases during the period of increased diuresis. The increase in kidney-volume is most marked at a point slightly earlier than the point of maximum increase in diuresis. At this point, too, the kidney vessels appear to be widely dilated, judging by the great increase in pulse-volume shown in the oncometer tracing: and this at a time when the general blood-pressure shows no change. This renal vaso-dilatation gradually fades away as the increase in diuresis is passing off.

With regard to the contradiction between the results of various observers as to the effect on diuresis produced by injecting saline solution intravenously the following points occur to me as worthy of notice:

1. The majority of these observers have injected enormous quantities, many of them having allowed the fluid to flow slowly into the circulation, thus Frey (*loc. cit.*) injected 1000 c.c., the period of injection being spread over 45 minutes. In such cases I would suggest that it is possible that the increase in diuresis which they have observed is really nothing more than what I have described as the immediate effect, and the fact that it is more prolonged than in my experiments is due to the prolonged period of injection; that it is in fact the sum of a series of "immediate effects" blended into one. When injecting smaller amounts, Frey's results agree with mine in that the diuretic effects were transient.

2. When such large volumes of fluid are injected into an animal, volumes which must be regarded as unphysiological, seeing that no animal would normally take in an amount of fluid approximating to or even exceeding its body-weight at any one time, some method other than a physiological method must be called into play in order that the animal may rid itself of the excess of fluid, so that it is not difficult to imagine that under these conditions the injection of saline may be followed by

the excretion of an increased amount of "urine," though it does not necessarily follow that this at all represents what takes place normally. The fact, too, that some of those observers who have injected these large amounts of saline have observed that the fluid which is excreted by the kidneys after these lavage experiments contains sugar, seems to lend support to the idea that some process other than the normal physiological process of diuresis is at work. In no case have I detected the presence of an increased quantity of reducing substance in the urine as a result of any of the injections which I have made. Thompson (*loc. cit.*) suggests that glycosuria is probably not produced until a large volume of fluid has been injected.

3. Of all the results of other observers which I have examined, those of Thompson (*loc. cit.*) are the only ones which to my mind present any difficulty in the way of compatibility with my results. He injected saline solutions intravenously and obtained in some instances what approximates to my delayed effect on the diuresis, an effect which I have been unable to obtain by the injection of saline, but only by the injection of extracts. It is true that Thompson's results were not all in agreement, though the majority gave a positive effect, whilst in my experience such a positive result is quite the exception. I can only suggest that either the intermittency of the anæsthetic may have produced an unusual result in Thompson's hands, or that the cause is to be sought in a possibly hyperfluid condition of the tissues of the animals which he used, an explanation which I put forward in my previous paper to explain a similar phenomenon. In this case there was substantial evidence that such was the true explanation.

So long a period elapses between the injection of these extracts of gastro-intestinal mucous membrane into the circulation and the production of the delayed effect on the diuresis that it seems probable that the extract itself is not directly diuretic, but that it acts as a hormone maybe for the production of a directly diuretic substance, which may be produced by one of the organs of internal secretion. At all events it would appear that, if this is not the case, the extract of gastro-intestinal mucous membrane must undergo some change in the circulation before it is capable of exciting the kidneys to increased activity.

Protocols of typical experiments.

1. Showing "immediate increase" due to injection of an extract of duodenal mucous membrane. Cat—urethane—vagi severed.

No. of drops of urine in successive minutes:

2, 2*, 1, 2, 6, 5, 4, 3, 2, 2.

* 12 c.c. extract of duodenal mucous membrane injected intravenously.

2. Showing "immediate increases" due to successive injections of normal saline solution. Cat—urethane—vagi severed.

No. of drops of urine in successive minutes:

2, 1*, 2, 4, 2, 2, 1—1, 1*, 3, 5, 3, 3, 2, 2.

* 10 c.c. saline solution injected intravenously.

3. Showing quantitative differences between "immediate effects" produced by saline solution and by extract of gastro-intestinal mucous membrane respectively. Cat—urethane—vagi severed.

No. of drops of urine in successive minutes:

1, 0, 1*, 0, 3, 2, 2, 1, 1—2, 1†, 2, 3, 6, 6, 5, 4, 3, 2.

* 10 c.c. saline solution intravenously.
† 10 c.c. extract of gastric and duodenal mucous membrane intravenously.

4. Showing "immediate increases" produced by two successive injections of extract of gastro-intestinal mucous membrane and by a following injection of saline solution. Cat—urethane—vagi severed.

No. of drops of urine in successive minutes:

1, 1*, 1, 3, 4, 2, 1—1, 1*, 3, 6, 6, 4, 3, 2, 1, 1—1, 1†, 5, 6, 4, 3, 3, 2, 1.

* 10 c.c. extract of gastric and duodenal mucous membrane intravenously.
† 10 c.c. saline solution intravenously.

5. Showing progressive diminution in the amount of urine excreted when no fluid is given. Cat—urethane—vagi severed.

No. of drops of urine in successive periods of 10 minutes:

36, 36, 34, 33, 26, 23, 19, 18, 16, 14, 13, 11, 10, 9, 8.

6. Showing "delayed increase" produced by injection of an extract of gastro-intestinal mucous membrane. Cat—urethane—vagi severed.

No. of drops of urine in successive periods of 10 minutes:

11, 10*, 16, 10, 12, 12, 13, 12, 12, 12†, 19, 14, 13, 14, 14, 17, 17, 26, 35, 34, 32, 28, 27, 28, 26, 25, 24, 22, 22, 21, 19, 18.

* 12 c.c. saline solution intravenously.
† 12 c.c. extract of gastric and duodenal mucous membrane intravenously.

7. Showing "delayed increase" produced by injection of extract of duodenal mucous membrane. Cat—urethane—vagi severed—eviscerated.

c.c. of urine in successive periods of 10 minutes:

0·92, 0·87*, 1·06, 1·10, 1·25, 1·48, 1·61, 1·96, 2·14, 2·21, 2·20, 2·24, 2·19, 2·19, 2·21, 2·21, 2·18, 2·20, 2·24, 2·08, 1·99, 1·97, 1·92, 1·93, 1·90, 1·89, 1·89, 1·82, 1·80.

* 12 c.c. extract of duodenal mucous membrane intravenously.

8. Showing "delayed increase" produced by injection of extract of duodenal mucous membrane. Cat—urethane—vagi severed.

c.c. of urine in successive periods of 10 minutes:

0·49, 0·43*, 0·60, 0·32, 0·39, 0·52, 0·48, 0·54, 0·61, 0·72, 0·63, 1·10, 1·27, 1·25, 1·39, 1·89, 1·88, 1·88, 1·81, 1·78, 1·96, 1·74, 1·65, 1·66, 1·34, 1·02, 1·00, 0·97, 0·98, 0·79, 0·78, 0·57, 0·49.

* 12 c.c. extract of duodenal mucous membrane intravenously.

9. Showing "delayed increase" produced by injection of extract of duodenal mucous membrane. Cat—urethane—vagi severed.

No. of drops of urine in successive periods of 10 minutes:

31, 30*, 43, 40, 36, 31, 39, 52, 62, 51, 61, 50, 31, 25, 23, 22, 20, 19, 18.

* 12 c.c. extract of duodenal mucous membrane intravenously.

10. Showing "delayed increase" produced by injection of extract of duodenal mucous membrane. Cat—urethane—vagi severed—eviscerated.

No. of drops of urine in successive periods of 10 minutes:

8, 8*, 14, 12, 15, 16, 17, 19, 25, 30, 26, 22, 18, 16, 14, 15, 12, 10.

* 12 c.c. extract of duodenal mucous membrane intravenously.

11. Showing "delayed increase" produced by injection of extract of duodenal mucous membrane. Cat—urethane—vagi severed—eviscerated.

No. of drops of urine in successive periods of 10 minutes:

18, 17*, 24, 15, 15, 17, 22, 28, 27, 23, 25, 24, 23, 23, 22, 21, 22, 25, 26.

* 12 c.c. extract of duodenal mucous membrane intravenously.

SUMMARY.

1. A new form of bladder-cannula is described.

2. When experimenting on unanæsthetized animals the diuretic action of a substance may be masked by reflex or other causes.

3. The mucous membrane of the stomach, duodenum, jejunum, and ileum contains a diuretic substance: that obtained from the duodenal mucous membrane is the most active.

4. This diuretic substance can be extracted in water (or salt solution) either at room temperature or boiling, and the extraction is independent of the presence of acid. The most active preparations have been extracted in acidulated salt solution at room temperature.

5. Subsequent boiling does not destroy the activity of these extracts.

6. These extracts lose their activity on keeping.

7. Intravenous injections of small amounts of normal saline solutions produce an immediate increase in diuresis: this increase is but transient and is very small in amount.

8. Intravenous injections of small amounts of extracts of gastro-intestinal mucous membrane produce (*a*) an immediate increase in diuresis, similar to that produced by injections of saline, and (*b*) a delayed increase, which is considerable in amount and comparable to the increase in diuresis produced by giving water by the mouth to normal animals. This delayed increase is not produced by intravenous injections of physiological amounts of saline.

9. There is sufficient evidence that the physiological process of diuresis depends not only on the integrity of the kidneys and circulatory system and on the introduction of a certain quantity of fluid into the organism, but also on the presence of a specific substance contained in the gastro-intestinal mucous membrane; which substance is, presumably, taken up by water during the process of absorption from the gastro-intestinal tract.

REFERENCES.

(1) **Barcroft** and **Straub.** This Journal, xli. p. 145. 1910.
(2) **Bock** and **Hoffmann.** Arch. f. (Anat, u.) Physiol. 1871, p. 550.
(3) **Cow.** Arch. f. exp. Path. u. Pharm. lxix. p. 397. 1912.
(4) **Dastre** and **Loye.** Arch. de physiol. 1889, p. 253.
(5) **Frey.** Pflüger's Arch. cxii. p. 71. 1906; cxx. p. 93. 1907.
(6) **Ginsberg.** Arch. f. exp. Path. u. Pharm. lxix. p. 381. 1912.
(7) **Hamburger.** Ztschr. f. Biol. xxvii. p. 259. 1890.
(8) **Heidenhain.** Pflüger's Arch. xlix. p. 209. 1891.
(9) **Limbeck.** Arch. f. exp. Path. u. Pharm. xxv. p. 68. 1888.
(10) **Magnus.** Ibid. xliv. p. 68. 1900.
(11) **Sobieranski.** Ibid. xxxv. p. 144. 1895.
(12) **Stern.** Ibid. xix. p. 89. 1885.
(13) **Thompson.** This Journal, xxv. p. 487. 1900.

ON THE NATURE OF THE FUNDAMENTAL ACTIVITY OF THE NERVOUS CENTRES; TOGETHER WITH AN ANALYSIS OF THE CONDITIONING OF RHYTHMIC ACTIVITY IN PROGRESSION, AND A THEORY OF THE EVOLUTION OF FUNCTION IN THE NERVOUS SYSTEM.

By T. GRAHAM BROWN (*Manchester*).

CONTENTS.

PAGE
I. Experiments in which the spinal cord is cut . . . 24
II. Experiments in which the peripheral reflexes are tested during narcosis progression 27
III. Movements of narcosis progression after de-afferentation of one hind limb 32
IV. The general bearing of these experiments . . . 33
V. The fundamental conditions of rhythmic progression . 34
VI. The nature and position of the reflex 39
VII. Evolution of function in the nervous system . . . 43
Summary 44

Introduction. The spinal reflex holds such undisputed sway in thought as the fundamental activity—the "functional unit"—of the nervous system that the time appears an appropriate one for the examination of the foundations of its supremacy[1].

The reflex is usually looked upon as consisting, first, in a centripetal process which passes towards the neuraxis in the afferent part of the arc and is there reflected by the mechanism of the grey matter into, secondly, a centrifugal process which travels in the efferent part of the arc.

What we term the "spinal reflex" is a conception in part morphological and in part functional. For it is an activity which is propagated in the animal from periphery to periphery through the mediation of the "reflex arc." That arc itself is usually looked upon as built up of various subsidiary morphological units—the receptive organ, the afferent neurone, one or more interposed neurones, the efferent neurone, and the effector organ—and the whole arc ministers to the functional unit only in as far as its constituents subserve the common function of the transmission of a state of changed activity from one end to the other.

---

[1] Until comparatively recently the nerve-cell with its various morphological processes and the activities of this system were together regarded as the "functional unit" of the nervous system. Sherrington, in the "Integrative Action of the Nervous System" (1906), appears to have first defined the *reflex* as the functional unit.

Of course this conception is an artificial and a crude one. Every afferent path tends, in its activity, to activate many efferent paths; and every efferent path is activated by many afferent paths. But it is important to note that in the conception as it is usually accepted the afferent and efferent paths of the morphological arc, and the centripetal and centrifugal activities of the functional process, are given equal values.

With this conception of a functional and morphological unit as an instrument various speculations with regard to the evolution of the nervous system have been put forward; and various speculations have also been made concerning the manner in which its more complex activities—for instance that of progression—are built up.

Thus a difficulty in the problem of the evolution of the nervous system—a difficulty presented by the idea that the afferent and efferent portions of the arc have equal values—arises when it is attempted to account for the manner in which the afferent and efferent neurones appeared simultaneously in the course of that evolution; and, as is well known, it has been surmised that at first a mechanism similar to the neuro-muscular cell arose and that this unit—which performs the functions of receptor, conductor, and effector—became subdivided into more specialised constituent units each of which performed in the arc its own more specialised function. Opposed to this idea is that put forward by the Hertwigs that the effector cell, the receptor cell, and the intermediate ganglion cell evolved side by side and in their interdependent evolution at length attained to the complex reflex arc. But more recently G. H. Parker[1] has advanced the theory that the effector organ (primitive muscle cell) first evolved and was later joined by the receptor and by the intermediate neurone to which he gives the name "adjustor."

When we turn from the problems of functional evolution to those of functional analysis of the most complex activities evolved the dominance of the reflex idea is still apparent. Thus in such an activity as progression in the mammal it is evident that any one limb performs the acts of flexion and of extension rhythmically repeated. In the light of the view that the evolution of function in the nervous system has been upon the lines of the building up of complexes out of the fundamental units of activity—the reflex unit activities—what more natural than to attempt to analyse such a complex as that of mammalian progression in terms of the more simple reflex activities which may be investigated? Thus—in a crude formula—the act of progression in a limb consists in a rhythmic alternation of the activity which we know as the flexion-reflex and the activity which we know as the extension-reflex.

---

[1] *Proc. Amer. Phil. Soc.* L. p. 217. 1911.

Granted the assumption that progression is compounded of the reflex activities of flexion and extension there are two species of these reflexes either or both of which might be the proper components—the extero-ceptive reflexes (those evoked by stimulation of the integumentary receptors) and the proprio-ceptive reflexes (those evoked by stimulation of the deeper receptors in the muscles, tendons, and joints).

Philippson, who was the first really to investigate the phenomenon of progression from its functional aspect[1], emphasised the importance of the extero-ceptive reflexes, but **Sherrington**[2] has shown certain objections to this view and himself laid stress upon the proprio-ceptive mechanism in the act of progression.

The present author[3] was, however, able to demonstrate for the act of progression which may be evoked in the hind limbs on rapid division of the spinal cord in the decerebrate cat that it might appear even after destruction of the proprio-ceptive mechanism and of the extero-ceptive mechanism of both hind limbs. This experiment shows firstly, that if the act, as regards a hind limb, is compounded of reflex activities the afferent paths of these do not necessarily lie in the same limb, and secondly, that the rhythmic alternation of activities is not conditioned by afferent self-generated stimuli which have their origin in either hind limb—or for that matter in either fore limb.

In that same paper the author showed that the phenomena which follow this rapid division of the lower thoracic spinal cord enter with a state of maintained flexion and end in a state of maintained extension. In the intermediate phase—where there is a more or less equal balance of the two activities—the rhythmic act of progression occurs. As this experiment of necessity eliminates the possibility of a peripheral "Selbststeuerung" the problem of the nature of that rhythm at once arose and the suggestion was put forward that progression was conditioned during this state of "balance" of the two opposed activities (flexion and extension).

---

[1] *L'autonomie et la centralisation dans le système nerveux des animaux*, Bruxelles, 1905.

[2] *Journ. Physiol.* XL. p. 28. 1910.

[3] *Roy. Soc. Proc.* B, LXXXIV. p. 308. 1911.

In another paper[1] it was found for another phasic phenomenon, that of "rhythmic rebound," that there was again evidence of the balance of two antagonistic factors; and later A. Forbes[2] and the author[3] demonstrated simultaneously that rhythmic reflex movements might be evoked during the synchronous application of two antagonistic reflex stimuli. The author showed that the antagonistic reflex stimuli must be nearly balanced, for graded relative increase in the strength of one was accompanied by gradual disappearance of the rhythmic phenomenon; that the phenomenon might be evoked in de-afferented preparations; and that it might occur in the low spinal preparation, and therefore that the rhythm was conditioned in the local lumbar centres. Sherrington[4] more recently has confirmed these observations that a rhythmic activity akin to the phenomenon of progression may be evoked on synchronous application of two nearly balanced antagonistic stimuli.

The problem of progression as it occurs in the mammal now resolves itself into this:—rhythmic progression is conditioned by an equal balance of two antagonistic central activities: are these fundamentally of peripheral origin?

It might thus be supposed that in normal progression the phenomenon is fundamentally conditioned by a nearly equal balance of the activities of peripheral flexion-producing stimuli and of peripheral extension-producing stimuli; but that the afferent neurones which condition these need not necessarily enter the cord in the same region (for instance, the lumbar) as that to which the limbs examined belong. Thus the phenomenon of progression evoked by rapid division of the lower thoracic spinal cord even after complete de-afferentation of the hind limb might be produced by the mechanical stimulation in the cord of descending fibres some of which give the flexion stimulus and some of which give the extension stimulus.

Or it might be supposed that the fundamental conditioning of the act is not of this nature but that a balance of these antagonistic activities may reinforce it; that the equal balance of the antagonistic stimuli reproduce in the centres a state similar to that which fundamentally conditions the act, but that the fundamental condition is not the balance of peripheral activities.

---

[1] *Quar. Journ. Exp. Physiol.* IV. p. 331. 1911 (see p. 393).

[2] *Roy. Soc. Proc.* B, LXXXV. p. 289. 1912.

[3] *Ibid.* B, LXXXV. p. 278. 1912.

[4] *Ibid.* B, LXXXVI. p. 233. 1913.

The author[1] has already mentioned (but without detail) some experiments which throw light upon this problem. As they appear to have an important bearing not only upon this point but also upon some other problems of the nature of the activities of the nervous system, this paper is devoted to their description.

Methods. The phenomenon of progression here examined is that which sometimes occurs in cats while under the influence of a general anæsthetic—"narcosis progression." In this phenomenon walking, running, or galloping movements may occur in all four limbs. The phenomenon may commence in the stage of light narcosis and then the movements as a rule are of large amplitude. If the narcosis is gradually increased in depth the movements gradually decrease in amplitude until they finally disappear altogether. The phenomenon may also commence in the stage of deep anæsthesia and then the movements are of small amplitude. Further increase in the depth of narcosis makes then disappear as before, but if the depth of narcosis be decreased they gradually increase in amplitude and finally stop suddenly when at their full extent. The phenomenon usually occurs, if it occurs at all, shortly after the commencement of narcosis and is difficult to induce after the animal has been anæsthetised for more than half an hour.

The movements have been recorded both in the intact limbs and in the isolated muscles. The procedure is as follows:—

The cat is put under general narcosis and at once the skull is trephined for subsequent decerebration. The spinal cord is exposed in the lower thoracic region, a loose ligature is passed behind it, and the wound is closed. A tube is placed in the trachea and the carotid arteries are ligatured (sometimes the ligatures are not tied). All the muscles acting upon either hip-joint are then functionally destroyed by motor paralysis (division of their tendons of insertion, division of the ham-string nerve, division of the motor branches of the femoral nerve, division of the obturator nerve). This also destroys the muscles which act upon the knee-joints. In the left hind limb (that the muscles of which are not recorded) the great sciatic nerve is divided—thus destroying all the muscles of the leg and foot. In the right hind limb the muscles of the leg are destroyed by motor paralysis except tibialis anticus and gastrocnemius-soleus (or soleus alone) and the foot is amputated. The cat is then laid upon its back with its right thigh perpendicular to the table and its right leg pointing to the recording levers. That leg is fixed by means of steel drills through the lower end of the femur and through the lower end of the tibia. The retained muscles are attached to the recording levers by means of long threads, and tripolar electrodes are placed upon the two long saphenous nerves.

---

[1] *Roy. Soc. Proc.* B, LXXXVI. p. 150. 1913.

It is essential for the success of these experiments that this procedure be performed as rapidly as possible. With practice the whole preparation may be completed—and movements recorded—within 25 minutes of its commencement. In very favourable circumstances the time of preparation may be reduced below 20 minutes, but even then the phenomenon occurs with comparative rarity after the individual muscles have been prepared for recording.

When the movements occur the spinal cord may rapidly be divided by opening the wound in the back, drawing upon the loose ligature, and snipping the cord across with a sharp pair of scissors. Before this is done the rhythmic progression may be interrupted by peripheral stimulation (rapid faradic shocks) applied to the saphenous nerves.

Sometimes the movements may commence during the preparation of the animal. Clips attached by threads to the recording levers are kept in readiness, and so is a steel bar fixed in a stand so that it is parallel to the table at a height of about 4 cm. The cat is at once laid prone with a hot water bottle under the abdomen, the bar is slipped under the two ankles, and the clips are attached to the pads of the feet. The movements may thus be examined in the intact hind limbs within a few seconds of their first appearance; and later the movements may occur—and be recorded—after the preparation of the individual muscles.

To prevent any possibility of mistake it must be clearly stated that in all these experiments and for the whole of each of them the animals (cats) were completely unconscious and remained so until they were destroyed at their terminations. As the phenomenon of narcosis progression may persist through varying depths of narcosis I have used the terms "comparatively light narcosis" and "comparatively deep narcosis." The former means "a certain depth of narcosis at which, the cats being unconscious, the movements of narcosis progression tend first to appear as the depth of narcosis is gradually increased and at which these movements then exhibit their greatest extents in the flexor beats." The latter term means "that depth of narcosis at which the movements tend to be abolished when the depth of narcosis is progressively increased."

I. EXPERIMENTS IN WHICH THE SPINAL CORD IS CUT.

In these experiments the movements were observed in the intact hind limbs alone and, except in one instance, not in the isolated muscles.

If the spinal cord be severed rapidly when the movements of narcosis progression are in being during "comparatively light narcosis" it has been observed that after one more "beat" there may occur complete cessation for a period of 12 seconds or more. But thereafter very small movements may again appear. In the experiment in which this was observed the narcosis progression was examined as it occurred in the individual muscles, and it was found that the slight movements after division of the cord were of a rate of about 0·8 "beats" per second—whereas the movements of large extent which were in being at the time of division of the spinal cord were of a rate of about 1·8 "beats" per second.

In another experiment the narcosis was also comparatively light at the time the spinal cord was cut. The movements of narcosis progression were rather irregular in their appearance—that is to say that for short intervals of time they disappeared. When they occurred with regularity their rate was one of about 0·9 "beats" per second. At the time at which the spinal cord was divided the movements were decreasing in extent, and the narcosis was probably becoming deeper. Division of the cord was followed by flexion movements in the two hind limbs. These were of small extent and duration and were immediately followed —1 second after the division—by movements of narcosis progression. The "beats" were greater in extent than before the division of the cord. At first they were of the same rate of rhythm. They increased in extent for about five seconds, and then their rate increased markedly until it was one of about 1·8 "beats" per second. About 28 seconds after division of the spinal cord the beats again became slower in rate and the phenomenon again became irregular. Cessation of the movements occurred abruptly and the final appearance of the record was in appearance similar to that before division of the spinal cord, with the exception that the movements were of greater extent.

In another experiment, where the depth of narcosis as judged from the extent of the "beats" in the narcosis progression phenomenon was deeper, similar results were obtained. Here division of the spinal cord was followed by a state of maintained flexion during which the movements of progression at first were abolished but later re-appeared. They were then of very much greater extent than before. Here the rate of rhythm changed from one of 1·2 "beats" per second before division of the spinal cord to one of about 2·0 "beats" per second after that division.

In a fifth experiment the movements of narcosis progression were in being during deep narcosis. The spinal cord was divided and there occurred scarce a movement in either hind limb. The rhythmic movements were, however, abolished for a space of about 16 seconds. They then suddenly re-appeared and were then of greater extent than before but of about the same rate of rhythm—1·4 "beats" per second as compared with 1·2 "beats" per second. Here the movements gradually became smaller and finally faded away. The small movements which followed the rapid division of the spinal cord in this experiment were not of so great extent as that of the "beats" which were at the moment in being.

In a final experiment (Fig. 1) the narcosis progression phenomenon was in being at a very great depth of narcosis and the "beats" were of very small extent but at the same time were very regular in rate and extent. They were accurately alternating in the two hind limbs. The spinal cord was divided and the division produced scarce a movement in either limb. There was no interference with the rhythmic movements. There was a very slight slowing of the rhythm—from a rate of about 1·2 "beats" per second to one of about 1·1 "beats" per second. The "beats" became slightly greater in extent and lasted for about 75 seconds after the division of the spinal cord—gradually fading away. The slight variations in rate and extent of the "beats" in this experiment were not greater than those which may occur during records in which there is no interference with the spinal cord and in which the depth of narcosis probably is slightly varied.

These experiments seem to show that if the spinal cord is rapidly divided when the movements of narcosis progression are in being at a comparatively slight depth of narcosis, there is usually first a phase in which the phenomenon is abolished and then a phase in which the beats re-appear and are of greater extent and rate of rhythm than before.

They also seem to show that the movements may occur at a depth of narcosis at which the rapid division of the spinal cord produces little or no reaction, and that they may then persist almost unchanged.

At this depth of narcosis the phenomenon is therefore one which may be exhibited in the lumbar spinal centres alone, and is then not conditioned by the activity of higher centres.

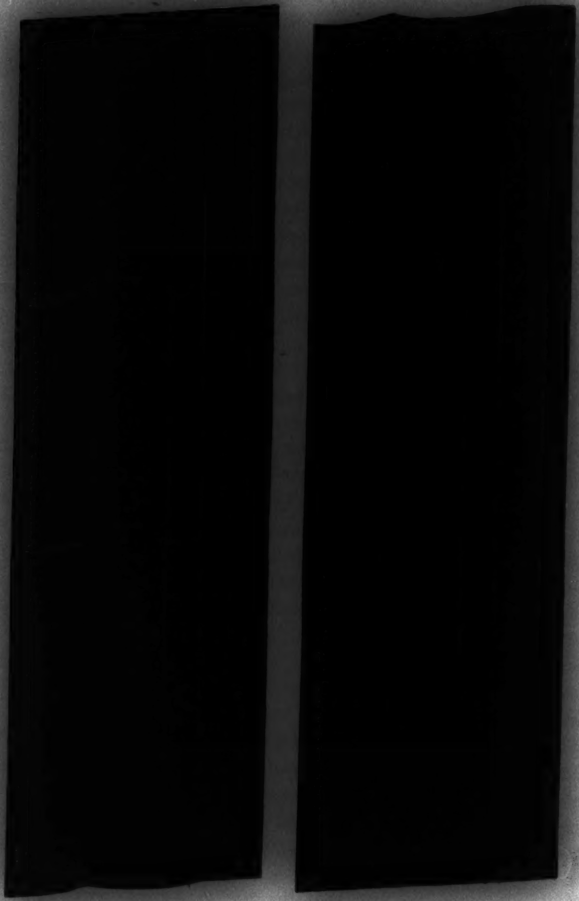


Fig. 1. Normal cat under deep narcosis, a record of the movements of "narcosis progression" in the intact hind limbs. Upper line registers the movements at the left ankle joint and lower line registers those at the right ankle. In either case the rise of the curve denotes flexion and the fall extension. Time in seconds. On the record a millimeter scale was drawn before varnishing, and is therefore

reduced in proportion with the record. The two parts of the figure are directly continuous.

The narcosis movements registered are of small extent. It will be observed that they are almost absent in the right limb. The movements of flexion in the two limbs are, however, seen to be alternate. At one point in the record ordinates have been drawn to illustrate this. These are numbered 2, and 3. It will be seen that between 2 and 3 there occurs a pause in the upper (left) curve and a movement of flexion-extension in the lower (right) curve.

At the point marked by ordinates x, x' on the two curves the lower thoracic spinal cord was rapidly severed between segments *XI* and *XII*. This severe flexion-producing stimulus produces of itself little movement in the two curves. Thereafter the beats of the narcosis progression gradually increase in height but, after reaching a maximum, gradually decrease again and finally fade away.

This figure demonstrates in the first place that the phenomenon of narcosis progression may be one which occurs in the local centres of the lumbar segment of the spinal cord and does not depend upon impulses from the higher regions of the neuraxis. Secondly it seems to show that the phenomenon may appear at a depth of narcosis at which the spinal reflex is put out of action; for the stimulus of division of the spinal cord is usually a much stronger one than any peripheral flexion-producing stimulus, and here even it appears to be almost valueless.

As rapid division of the lower lumbar spinal cord is—in the decerebrate cat—one of the strongest, and probably the strongest, of flexion-producing stimuli it is probable that the phenomenon of narcosis progression may occur when the peripheral reflexes have been abolished by a general chemical narcotic.

II. EXPERIMENTS IN WHICH THE PERIPHERAL REFLEXES ARE TESTED DURING NARCOSIS PROGRESSION.

Further to test the possibility that the phenomenon of narcosis progression may occur at a depth of narcosis at which the peripheral reflexes are abolished, the movements have been examined as they occur in the individual muscles of the leg and reflex stimuli have been then applied to afferent nerves of the same and of the contralateral hind limbs.

These stimuli should usually evoke the ipsilateral flexion-reflex and the contralateral extension-reflex respectively, but as a matter of fact it has been found that the contralateral extension-reflex has always been inexcitable at any depth of narcosis at which the movements of narcosis progression occur at all—and this even when the strongest stimuli which may be given with the coils in use are applied. In the same way it has been observed that extensor rebound ("extensor rebound contraction after inhibition") in the flexion-reflex has been absent.

The ipsilateral flexion-reflex has been found to occur at the depth of "comparatively light narcosis" at which the phenomenon of narcosis progression is of great extent. This reflex then appears often to be peculiarly characterised by a tendency to flexor after-discharge. This tendency is greater than in the flexion-reflex of the decerebrate and low spinal preparations, and if the sudden terminal relaxation of the flexor muscles in the flexion-reflexes of these conditions be regarded as the reciprocal of the "extensor rebound contraction after inhibition," which is the most common positive terminal phenomenon in these reflexes, it seems by no means unlikely that the presence of this tendency to flexor after-discharge is associated with the absence of extensor rebound contraction in the state of general chemical narcosis.

In these experiments, although a contralateral extension-reflex has never been observed in the state of narcosis, the contralateral stimulus has not infrequently been observed to evoke the abnormal contralateral flexion-reflex. This has occurred in a greater proportion of experiments than that in which it is seen in the ordinary decerebrate preparation, and in the individual experiments in which it has occurred during chemical narcosis it has not seldom been found that later, when the preparation had been converted to the decerebrate condition and the effects of narcosis had been allowed to pass off, the contralateral reaction was the normal extension-reflex. These abnormal contralateral flexion-reflexes under narcosis have been followed by marked terminal flexor after-discharge.

This occurrence of an abnormal contralateral flexion-reflex in the state of narcosis is a point of interest. It might be argued that the disappearance of the extension-reflex, and perhaps also of the extensor rebound in the flexion-reflex, in chemical narcosis is due to a paralysis of those interconnexions between the two sides of the spinal cord which condition the related acts of the pair of hind limbs. That this might be so is also suggested by the observation that in the decerebrate preparation when one lateral half of the lumbar spinal cord has been removed the ipsilateral flexion-reflex shows a marked tendency to flexor after-discharge—often accompanied by the reciprocal "extensor rebound relaxation after inhibition[1]." But that the chemical narcotic does not act as it were by producing a longitudinal split of the two halves of the lumbar segment of the spinal cord is shown by this observation, that the abnormal contralateral flexion-reflex may occur although the normal contralateral extension-reflex does not; and by the additional observation that even at a great depth of narcosis when the narcosis progression is occurring after division of the spinal cord in the lower thoracic region the movements of the two hind limbs are accurately alternate, and remain so for considerable lengths of time.

---

[1] *Quar. Journ. Exp. Physiol.* v. p. 233. 1912.

Comparatively light narcosis. When the movements of narcosis progression are of good extent and are in being when the depth of anæsthesia is "comparatively light" the ipsilateral stimulus evokes a reaction of flexion and this interferes with the phenomenon of progression. The flexor contraction in the reflex is then at first often of greater extent than the flexor contraction in the "beat" of the progression phenomenon. If the stimulus be prolonged in time the flexor contraction tends to die away and at the same time irregularities which correspond to the progression "beats" appear upon it. In other words, at first the reflex-flexion completely abolishes or masks the "beats" of the progression flexion, but later—as the reflex-flexion diminishes in value—the "beats" begin again to break through, or to be unmasked. If the reflex stimulus is of short duration in time there may persist a flexor after-discharge which has a similar appearance to that of the diminishing reflex-flexion described above. After such a reflex-flexion there appears sometimes to occur a quickening of the rhythm of the "beats" of the narcosis progression phenomenon.

When brief ipsilateral stimuli are applied at different points in the cycle during a period in which the phenomenon of narcosis progression is occurring steadily there appears to be evidence of a phase of relative refractability in each cycle, but there is no evidence of an absolute refractory phase when moderate stimuli are used.

While narcosis progression is in being in the stage of "comparatively light narcosis" the contralateral stimulus may give the same phenomena as the ipsilateral, and this even when the contralateral stimulus produces no response when the movements of narcosis progression are not in being. Very strong stimuli must be used in order to obtain the abnormal contralateral flexion effect.

Occasionally, however, when the narcosis is comparatively deep the effect of the contralateral stimulus may be to cause inhibition of the beats of the narcosis progression. A brief contralateral stimulus applied in the phase of flexor relaxation between two consecutive "beats" completely suppresses the second. The effect of this inhibition appears nevertheless to be transient, for the next "beat" in the series may be of the usual extent and the rhythm of the whole phenomenon is not "thrown out of step"—all that happens is that an individual "beat" fails to occur. If, however, the contralateral stimulus be of longer duration one "beat" may be completely suppressed and the following "beat" may be delayed in its appearance.

Deep narcosis. When the phenomenon of narcosis progression is in being and the depth of narcosis is progressively increased the progression movements may continue at a depth of narcosis at which the animal is almost killed. At the stage at which the progression movements are finally abolished the respiratory centre may also be paralysed, but more commonly it continues to be active at a slightly greater depth of narcosis than that at which the progression movements cease.

At that depth of narcosis at which the progression movements are only just in being, and indeed at considerably less great depths of narcosis, the contralateral stimulus is ineffective even when the maximum strengths of stimuli are applied. There then occurs no change whatsoever in the rhythmic progression phenomenon.

The effect of the ipsilateral stimulus seems to persist at a greater depth of narcosis than does that of the contralateral stimulus, but it too appears to be inoperative at the greatest depths of narcosis at which the progression phenomenon occurs.

In one experiment (Fig. 2) the phenomena of narcosis progression were recorded for a period of about 370 seconds. They started in the stage of comparatively light narcosis and during the record the narcosis was gradually increased in depth until the movements were almost abolished. A contralateral stimulus of maximum strength was used throughout the series and its effect upon the rhythmic progression was tested at intervals. At first it appeared to inhibit the flexor "beats," but long before the progression had been reduced to its minimum the contralateral stimulus became completely ineffective.

In the early stages of the record the ipsilateral stimulus applied was a comparatively weak one. It was given in the same manner as the contralateral at intervals during the rhythmic narcosis progression phenomenon. As the depth of narcosis was increased the strength of ipsilateral stimulation was increased until it was maximal—a strength far greater than that which it is advisable to use in the normal decerebrate and low spinal preparations. While the extents of the "beats" of the progression phenomenon were still well marked although at a great depth of narcosis this great strength of ipsilateral stimulation, even when applied for periods of time as long as 5 seconds, gave scarce any evidence of interference. There was nothing corresponding to the flexor contraction of the flexion-reflex, and all that happened was

Fig. 2. Normal cat under deep narcosis. A record of the movements of right tibialis anticus (upper curve) and gastrocnemius-soleus (lower curve) in narcosis progression. The rise of either curve denotes contraction and the fall denotes relaxation of the recording muscle. During the record ipsilateral and contralateral long saphenous nerves are stimulated. The upper signal line gives the points of commencement and termination of contralateral (left) stimulation. These are denoted by the letters *A* and *B* and ordinates corresponding to these points have been drawn on the muscle curves (a, a'; b, b'). In a similar manner the lower signal line (*X*, *Y*) gives the temporal relations of ipsilateral (right) stimulation and the corresponding ordinates are marked x, x'; y, y'. The stimuli used are maximal. Time in seconds.

This tracing is the terminal portion of a long record. The first stimulus here reproduced (*G*) is a contralateral one and has no effect on the rhythmic beats of the flexor. The second stimulus (*XXXII*) is an ipsilateral one and during its application there appears to be a slight reduction in the extents of the flexor beats. At the point marked *I* (two ordinates are here drawn) the drum was stopped for a space of about 60″. During this interval the afferent nerves were rearranged on the electrodes. The interval of rest and the rearrangement of the electrodes was performed to counteract as far as possible any "fatigue" effect. When the drum was restarted it will be observed that the movements of narcosis progression were smaller than before. The beats, as judged in the intervals between stimuli, are less regular than in the first part of the tracing here reproduced. The contralateral stimuli (*K* and *L*) are again apparently without influence. So too are the ipsilateral (*XXXIII*, *XXXIV*) with the exception that there appears to be a tendency for the beats to fail during ipsilateral stimulation. It will be observed that throughout no contraction of the extensor occurs. At the end of this record the movements fade away.

After the end of this record the ipsilateral stimulus was repeated at intervals of two minutes, and not for four minutes after the termination of narcosis (which coincided with the termination of this record) did a flexor contraction in response to this stimulation appear.

This figure illustrates a case in which it appears that the rhythmic phenomenon of narcosis progression continued at a depth of narcosis at which not only the contralateral extension-reflex but also the ipsilateral flexion-reflex was abolished even in response to maximal stimuli.

that occasionally a "beat" would fail. In fact, this great strength of ipsilateral stimulation appeared to be ineffective at the depth of narcosis which then obtained.

But repeated stimulation of an afferent nerve, as in this experiment, may produce a phenomenon of fatigue. In order as far as possible to eliminate this possibility the drum was stopped for a period of one minute during which no stimuli were applied, and at the same time the electrodes were moved to a fresh and more central part of the peripheral afferent nerve which was stimulated. When the record was resumed the state of narcosis was even deeper and the rhythmic movements of the progression phenomenon, although they still were in being, were very slight. It was then found that maximal contralateral and ipsilateral stimulation had no effect, and that the ipsilateral stimulus evoked nothing of the nature of a flexion-reflex.

The narcosis movements were then abolished by a further slight increase in the depth of narcosis and the narcotic was thereafter at once stopped. The progression did not again appear as the depth of narcosis gradually decreased. The effect of the maximal ipsilateral stimulus was then tested at different intervals of time after the cessation of the narcotic—that is, at different levels of decreasing narcosis. Two minutes after the cessation of narcosis the stimulus was inoperative. Four minutes later a very slight flexion-reflex was evoked, and then with decreasing depth of narcosis became ever of greater extent. The contralateral stimulus throughout was ineffective.

I should like to refer again here to an observation already published[1] on the effect of removal of one lateral half of the lumbar spinal cord. In one experiment the left lateral half of the lumbar spinal cord was removed in a cat and afterwards movements of narcosis progression occurred in the right hind limb.

III. MOVEMENTS OF NARCOSIS PROGRESSION AFTER DE-AFFERENTATION OF ONE HIND LIMB.

In the paper just quoted[1] in which the movements of narcosis progression as they occur in the cat were described it was stated that after de-afferentation of one hind limb the movements of narcosis progression had not been observed. In these experiments the posterior spinal roots in the lumbar region of the spinal cord were divided immediately before the observation of the animal for narcosis progression and it is perhaps remarkable that the movements should have occurred in either hind limb (they occurred in the hind limb which was not de-afferented) after this exposure of the cord.

---

[1] *Roy. Soc. Proc.* B, LXXXVI. p. 140. 1913.

In a more recent experiment the whole of the posterior spinal roots upon the right side of the lumbar spinal cord with the exception of the first post-thoracic root were divided about 15 months before the animal was examined under narcosis. When then put under the general chemical narcotic a phenomenon of rhythmic narcosis progression appeared in the left hind limb—that not de-afferented. The movements were therefore unilateral and remained so for a period of about 50 seconds. During this period there were no movements in the de-afferented hind limb. When the movements ceased in the left hind limb there shortly appeared similar movements in the right (de-afferented) limb, and these in their turn were unilateral. After they had persisted for a period of 20 seconds the movements of narcosis progression became bilateral and strictly alternate.

IV. THE GENERAL BEARING OF THESE EXPERIMENTS.

These experiments seem to indicate that the phenomenon of narcosis progression may persist in the hind limbs at a depth of narcosis at which the lumbar spinal centres respond neither to the excessively strong stimulus which a rapid division of the lower thoracic spinal cord inflicts upon them, nor to maximal peripheral stimulation of afferent nerves of the hind limbs.

But in this rhythmic activity of narcosis progression the lumbar efferent neurones are in action. The chemical narcotic therefore appears to affect the afferent path of the lumbar centres (or the first synapse) before it affects the efferent mechanism.

At this depth of narcosis it does not, however, affect the nice alternation of movement of the narcosis progression phenomenon. In all the depths of narcosis at which the phenomenon occurs it has been found that the extensor muscle plays no active part. But the movements of flexion and extension are still conditioned by contraction and relaxation of the active flexor muscle when it is made to work against some external agent such as gravity or the resistance of an elastic band.

We may therefore say:—these experiments seem to show that a certain complex rhythmic movement may occur at a depth of narcosis at which the peripheral reflexes (or at least the ipsilateral extero-ceptive flexion-reflex and its ally the contralateral extero-ceptive extension-reflex) are abolished: and therefore that the spinal efferent neurones may show some degree of independence of the afferent neurones and some of the phenomena of reciprocal innervation when functionally separated from them.

If this thesis be sustained the experiments localise many of the functions of the spinal reflex arc in the efferent neurones—but not necessarily in the single neurone.

For in the first place the explanation of the rhythm in such acts as progression cannot be made by evoking peripheral centripetal impulses as the fundamental condition. The rhythm may best be explained as conditioned by some function of the interconnexion of efferent neurones[1].

If this be the case it becomes likely that the explanation of reciprocal innervation may best be made on the same basis.

Again, and on the assumption that the more recent neural mechanisms are the more easily paralysed by a general chemical narcotic, these experiments seem to suggest that the most primitive activity of the spinal centres is that rhythmic one which we now see active in narcosis progression. This suggestion at once brings the fundamental activity of the spinal centres into close parallelism with that of the respiratory centre—another mechanism which is peculiarly resistant to the effects of general chemical narcosis.

The bearings of this theory will be noticed later, but it will be observed that it tends to displace the reflex as the unit of activity in the spinal centres and to put in its stead a mechanism composed of efferent neurones. Perhaps the simplest unitary mechanism is one formed of the efferent neurones to two antagonistic muscles; and as an artificial conception that of two such efferent "half-centres" (that is, two efferent neurones to antagonistic muscles) and their mutual interconnexions may be suggested.

V. ON THE FUNDAMENTAL CONDITIONS OF RHYTHMIC PROGRESSION.

When it was first shown[2] that mammalian progression may occur in the lumbar segment of the spinal cord after complete de-afferentation of both hind limbs, and after severance of the lumbar cord from the more oral parts of the neuraxis, it was observed that the phenomenon of progression which is obtained on rapid division of the spinal cord in the decerebrate cat is especially evidenced during a state of the balanced activities of antagonistic extension and flexion.

---

[1] *Roy. Soc. Proc.* B, LXXXV. p. 278. 1912.

[2] *Ibid.* B, LXXXIV. p. 308. 1911.

Later, when it was demonstrated that rhythmic movements of the same rate of rhythm as those of progression can be induced in the decerebrate and in the spinal cat when two antagonistic flexion-producing and extension-producing stimuli of a certain degree of equality are pitted against each other[1], the inference was drawn that, as the one stimulus when applied "pure" gives an arhythmic flexion-reflex and the other gives an arhythmic extension-reflex but yet when applied together they give the rhythmic response, the rhythm is not an inherent function of the efferent neurone—that is, of the "half-centre"—but that it is a function of the interrelations of the "half-centres."

In other words, progression seems to be conditioned, by some function of their mutual relationship, during the equal and opposite activation of linked antagonistic "half-centres."

Sherrington[2], who has endorsed these observations that a rhythmic phenomenon akin to that of progression may be evoked by the appropriate balance of antagonistic stimuli, in a paper lately published in this *Journal*[3] examines the question of the conditioning of these rhythmic phenomena. Dismissing the possibility of peripheral "Selbststeuerung" by stimuli—either extero-ceptive or proprio-ceptive—self-generated during the movements of the limbs, he discusses the following possibilities:—

In the first place it is possible "to regard, on the analogy of the respiratory centre, the rhythm of the step-reflex as of essentially intrinsic origin; a natural and native rhythmic reply of the nerve cells to an inner stimulus such for instance as the blood. This is a view to which T. Graham Brown inclines....It is a view that demands careful attention, but, as it seems to remove stepping from the category of reflexes except in so far as concerns regulation, the present argument which proceeds on the assumption that the forms of stepping here dealt with are reflex would be led too far afield by its consideration now."

From what has been said above it will however be noticed that the author does not in fact regard the rhythm of the phenomenon as conditioned by the native rhythmic reply of nerve cells but by a native rhythm which is a property of the interconnexions of antagonistic nerve cells and is set up during an equal and opposite activation of them.

In the second place Sherrington examines and accepts the possibility that the step-reflex may be conditioned by balanced antagonistic activation of the spinal centres. This "demands the presence of two separately placed peripheral stimuli which may be continuous, and it invokes the intracentral facts of 'rebound' and 'fatigue.' The two stimuli must be of opposed effect and...of closely balanced intensity in their action on the centre."

---

[1] *Roy. Soc. Proc.* B, LXXXV. p. 278. 1912.

[2] *Ibid.* B, LXXXVI. p. 233. 1912; and *Quar. Journ. Exp. Physiol.* VI. p. 251. 1913.

[3] *Journ. Physiol.* XLVII. p. 196. 1913.

Sherrington therefore at present favours the view that the act of stepping is produced peripherally by balanced antagonistic stimuli in the presence of the intracentral factors of "fatigue" and "successive spinal induction."

But "narcosis progression" occurs when the peripheral reflexes have been abolished by a general chemical narcotic. Most certainly the effect of a contralateral stimulus (when the movements are examined in the individual muscles of one hind limb) is abolished at a depth of narcosis much more light than that necessary to abolish the progression movements. And the effect of the ipsilateral stimulus itself seems to be abolished while the progression movements still persist. There can be no possibility here of a balance of antagonistic peripheral stimuli.

And if the phenomenon of spinal "rebound" is ever seen in chemical narcosis it is never seen at the depths of anæsthesia at which the progression phenomenon occurs.

This phenomenon of progression is therefore not conditioned by peripheral stimuli, and the inference must be drawn that the fundamental conditions of progression are not of peripheral origin—progression is not fundamentally a "reflex" act.

But on the other hand, as has already been pointed out[1], the phenomenon cannot be regarded as the fundamental property of the discharge of the single centre (that is, of the single nerve cell or "half-centre").

If so, how is the phenomenon of progression conditioned? The following suggestion may be put forward:—

The rhythmic phenomenon of progression is fundamentally conditioned by the balanced activation of antagonistic and linked efferent centres (or "half-centres"), but this activation is not necessarily of peripheral origin—it may, in fact, be produced by conditions similar to those of the "blood-stimulus" in respiration.

On the assumption that the rhythm is conditioned by some property of the interconnexions of these "half-centres" during this state, what explanation of the manner of its production may be given?

---

[1] *Roy. Soc. Proc.* B, LXXXV. p. 278. 1912.

The following explanation is suggested[1]:—

The efferent neurone may be supposed not only to activate its effector organ through the mediation of its axone but also, by means of some other branch-fibre or side-channel, at the same time reciprocally to depress the activity of an antagonistic efferent neurone; and similarly that antagonistic neurone may be supposed to depress the activity of the first neurone as well as to activate its own effector. There is some morphological evidence that the efferent neurones of the spinal cord send back from their axones a collateral branch soon after the axone leaves the cell-body. [For simplicity the consideration of interposed neurones in the arc is avoided.]

In such a system there would be a phenomenon of mutual inhibition. When one neurone ("half-centre") is activated it depresses the other synchronously with the activation of its own effector. But that other "half-centre" when active depresses the first, and when it is depressed its depression of the first "half-centre" will be diminished in value. Therefore the activation of the first will increase still further, and so on. This may be termed the "progressive augmentation of activation" by the process of mutual inhibition.

In theory, if this arrangement exists, the smallest stimulus which acts upon a centre if sufficiently prolonged should give a maximal activation of one of the "half-centres"—for the only limit set is that of complete depression of the other "half-centre." In practice this does not occur, and a limiting factor must be assumed.

Such a factor might be a process of "fatigue" in the collateral depressory connexions. There is some evidence that it exists. This "fatigue" may be termed the factor of "depreciation of inhibition."

If it be assumed that this depreciation of inhibition is proportional to the duration of the process of progressive augmentation of activation and proportional also to the value of that process, it is possible to frame a theory of the nature of the rhythmic phenomenon in the linked antagonistic "half-centres."

Thus when the two antagonistic "half-centres" are activated in equal degrees and with an extreme nicety of equality it might be supposed that no rhythmic phenomenon would occur. But if the balance was upset in the slightest degree to either side the process of progressive augmentation of excitation would set in. The activation of one "half-centre" would rise and that of the other would fall until the temporal process of depreciation of inhibition set a limit to the augmentation. When this point was reached the state of the activity of the centres would remain constant except for the hypothetical process of depreciation of inhibition which is assumed to increase with duration of time. This increase of the depreciation of inhibition would therefore tend to decrease the state of relative activation of one "half-centre" attained by the progressive augmentation of activation. There would therefore set in the opposite process—that is, progressive augmentation of activation in the other direction. This again would attain its maximum and the element of depreciation of inhibition, now acting in the opposite direction, would again tilt the process of progressive augmentation—and so the rhythmic phenomenon might be explained.

---

[1] *Quar. Journ. Exp. Physiol.* v. p. 237. 1912; also, *Roy. Soc. Proc.* B, LXXXV. p. 278. 1912.

One point at first sight appears to present difficulty. How may it be supposed that the depreciation of inhibition—the "fatigue"—acts in opposite directions? This might be supposed to be conditioned in such an arrangement of the centres (or "half-centres") simply on the assumption that the depreciation was, in some degree, proportional to the strength of the inhibitory impulses. Thus if one "half-centre" is in maximal activity the process by which it inhibits the other "half-centre" will be subject in the greatest degree to the depreciation of its inhibitory impulses; but the process by which the second "half-centre" inhibits the first will be practically at rest in this relative state of the centres. As the process of progressive augmentation of activation sets in in the opposite direction depreciation will make its appearance and will gradually increase in value in the inhibitory branch of the second "half-centre" while it will gradually decrease and finally disappear in the inhibitory branch of the first "half-centre."

In this phenomenon of narcosis progression as it occurs at great depths of narcosis we are faced with the problem of how best we may explain the occurrence of the rhythm which is not conditioned peripherally and is not a native property of the cell-body. It seems to me that some such explanation as that offered above is the simplest and the most easy to accept.

It places the efferent neurone—or rather the linked pair of antagonistic efferent neurones or "half-centres"—in an independent position as the morphological substructure of the functional unit of the nervous system. Upon them and activating them may be supposed to play two kinds of stimuli. Of these the first and most primitive is that which corresponds to the "blood-stimulus" in respiration; and the second is the more recent stimulus conditioned by the afferent path of what we know as the spinal reflex. In the light of this view the primitive activity of the linked "half-centres"—of the morphological unit—is supposed to be rhythmic; but the rhythm is not looked upon as conditioned in regard to its period by metabolic factors. It is rather regarded as conditioned by the periodic blocking and unblocking ("fatigue"), at a point (synapse?) in the path of "mutual inhibition" of inhibitory impulses which may have a much faster rate of rhythm of their own, and one perhaps conditioned by such metabolic factors. And again, in the light of this view it will be observed that a mechanism for reciprocal innervation is assumed to exist between the efferent "half-centres" and not in the (or is not necessarily assumed to be confined in the) afferent neurones.

In other words, the view here advanced is that the functional unit in the nervous system is not the activity of the reflex arc as is usually assumed, but that it is the activity of a unit which we may term the "half-centre"—or, perhaps, the centre composed of linked antagonistic "half-centres." There remains the question of the manner in which the peripheral reflex should be considered.

VI. ON THE NATURE AND POSITION OF THE REFLEX.

One of the most fundamental properties of the spinal reflex is that of reciprocal innervation first described by Sherrington. Where one set of muscles in a reflex act is activated, the activity of a set of antagonistic muscles is reciprocally depressed.

The reciprocal innervation might be conditioned in the peripheral afferent neurones. For it is possible that stimulation of a mixed nerve trunk might set into activity some fibres which ultimately activate one set of muscles, and other nerve fibres which ultimately depress the other antagonistic set of muscles. Or, again, each nerve fibre activated might branch within the spinal cord and one branch might activate one centre while the other branch might reciprocally depress the other centre. This second view is that to which Sherrington inclines.

On the other hand the reciprocal innervation might be conditioned by some such process of mutual inhibition as that developed in the section of this paper immediately preceding the present one.

The phenomenon of narcosis progression demonstrates an alternation of antagonistic activities. Even although the extensor muscle does not demonstrate active movements, in the flexor muscle there occur phases of alternate flexor contraction and flexor relaxation—that is, flexion and extension; and this phenomenon is seen even at the great depths of narcosis at which the afferent mechanism appears to be paralysed.

At this stage in the phenomenon it is therefore probable that although certain attributes of reciprocal innervation are exhibited there is yet no afferent mechanism in being to condition them. If this be the case it appears to be more probable that the conditioning is performed in the manner already set forth in this paper—and not essentially by processes of antagonistic and reciprocal activation and depression in the peripheral afferent neurones. It is not of course intended to state that such an arrangement is not present. All that can be said is that a peripheral afferent mechanism is redundant if present and if the manner here suggested is correct[1].

The view here suggested therefore places the conditioning of reciprocal innervation, of rhythmic activity, and of the various central factors, in a mechanism of mutual inhibition which links efferent antagonistic "half-centres." And it suggests that the fundamental activity of this system is the rhythmic. In what light are we to regard the peripheral reflex?

---

[1] During the correction of the proofs of this paper the following argument has suggested itself to me as a weighty one in connexion with the question of the manner in which reciprocal innervation is ordered: As I have shown, when two antagonistic stimuli of appropriate strengths are pitted against each order a rhythmic phenomenon is conditioned. The rhythm is evidenced in each of the two antagonistic muscles, *and their movements are then accurately reciprocal.* On the hypothesis of the ordering of reciprocal innervation by a mechanism of "mutual inhibition" between the antagonistic "half-centres" no difficulty is presented by the reciprocal relations of the rhythmic movements of the two antagonists. A difficulty is, however, presented if the attempt is made to explain the rhythmic phenomenon by the postulation of an interference which lies in a more peripheral point in the reflex arc. Thus in the ordering of the specific form of rhythmic activity here referred to it might be supposed that an influence which activates the flexor "half-centre" is interfered with by an influence which depresses it, and that the site of this interference is a common synapse between afferent neurones and efferent neurone. In a similar manner a similar interference might occur at another synapse for the extensor "half-centre." The occurrence of such interference might well explain the rhythmic phenomenon which occurs in either "half-centre"; and if the rhythm is dependent in rate upon the relative values of activation and depression in each "half-centre," the *rates* of rhythm in the specific phasic activity here examined would be alike in the two "half-centres"—in the two antagonists. But the movements of the two muscles—or the discharges of the two "half-centres" (efferent neurones)—*would not necessarily be reciprocal.* The difficulty here presented is a somewhat serious one, but it may perhaps be overcome by assuming that the discharges of the two antagonistic centres commence in opposite senses, and that then the rates of rhythm in the two "half-centres" are so accurately equal that the reciprocal relationship of the discharges is retained throughout the whole of the remainder of the period in which the two antagonistic stimuli are applied. But then, why should the first discharges be ordered reciprocally when the two antagonistic stimuli are synchronously commenced? In these circumstances the rhythmic movements of the two antagonists are still accurately reciprocal.

One peculiarity of the spinal reflex is that under certain conditions the activity may change over into the opposite direction even during the application of a constant stimulus[1]. Thus a flexion-reflex may change over into one of extension during the application of an evocative stimulus.

Again, if the state of the centres be tested during a period of stimulation by means of the application of a brief antagonistic stimulus it may be observed that the "background" reaction has an ever less value as the period of activity is prolonged—even although it may not actually reverse in its activity[2].

These and similar phenomena suggest that even in the peripheral reflex a process similar to the alternation of activity in progression occurs, although its period may be very much greater than the period of the rhythmic alternation in progression.

It is difficult to avoid the conclusion that the peripheral reflex is nothing more nor less than the distorted "beat" of the fundamental rhythmic phenomenon. On this hypothesis it might be supposed that the peripheral afferent stimulus, as it were, captures one side of the pair of linked "half-centres." As long as the peripheral centripetal impulses impinge upon the centre there occurs a constant elevation of the activation of one side of the unit system, and this may be sufficiently great to prevent an actual reversal of activity while the stimulus is in being. But nevertheless after the maximum of activity is attained there may occur a deterioration which is conditioned by the process of depreciation of inhibition, although the deterioration may be a slower phenomenon than that in the rhythmic phenomenon. When the peripheral stimulus is stopped there is withdrawn from the centres a factor which serves in part to neutralise the ordinary depreciation of inhibition. There then occurs the sudden reversal of activity which is seen in the most usual form of "rebound" phenomenon.

In short, the peripheral reflex may be regarded but as an exaggeration of one or other phase of the fundamental rhythmic activity. When the reflex stimulus is weak there occurs less distortion than when it is strong. There is then a less prolongation of the positive phase upon which the stimulus acts, and reversal of activity may even occur within the limits of stimulation. But when the stimulus is strong there occurs a greater prolongation of the positive phase and there may occur no reversal.

---

[1] *Quar. Journ. Exp. Physiol.* v. p. 237. 1912.

[2] *Ibid.* VII. p. 245.

The peripheral stimulus may be looked upon as acting upon the centre in a manner similar to the action of what may be termed the "blood-stimulus" but in a more discriminating way. For whereas it is possible that such a factor as the "blood-stimulus" would act equally upon the two antagonised "half-centres," the peripheral stimulus may be looked upon as activating principally (if not entirely) one only.

If these suggestions are correct the peripheral reflex cannot be looked upon as the functional unit in the activity of the nervous system. The afferent path is only one of the manners in which the unit centre may be activated. The reflex is not the unit by the combinations of which such complexes as those of progression are built up; the afferent path merely selects for activation some of the activities of the more fundamental phenomenon of rhythmic action. If so, what part do the peripheral reflexes play in the normal activity of progression?

Sherrington[1] at one time suggested that perhaps the act of progression is conditioned by the interference of a constant activity through the mediation of peripheral impulses self-generated in the moving limbs, and of these he laid stress upon the proprio-ceptive impulses which are engendered by the active contraction of the activated muscles. Fascinating as this suggestion is it does not give the fundamental conditions, for progression occurs after such impulses are eliminated.

But there is of course no doubt that such impulses exist in the normal condition—as his work has so clearly shown. What is their effect?

One of their possible functions, as has already been suggested[2], may be not to interfere with the maintained activation of the centres but actually to conserve it. And in this connexion Sherrington's recent observation[3], that when the muscles are de-afferented it is less easy than in the normal condition to obtain a maintenance of the rhythmic phenomenon which is conditioned by balanced antagonistic stimulation, is of interest. It is indeed possible that the self-generated proprioceptive impulses serve to keep balanced the activities of the two linked "half-centres," and especially that they serve to maintain this balance where by the accidents of the environment the individual steps of mammalian progression have to be varied in order to carry the animal with accuracy over an uneven surface.

---

[1] *Journ. Physiol.* XL. p. 28. 1910.

[2] *Quar. Journ. Exp. Physiol.* VI. p. 246. 1913.

[3] *Journ. Physiol.* XLVII. p. 196. 1913.

VII. ON EVOLUTION OF FUNCTION IN THE NERVOUS SYSTEM.

It is therefore possible to look upon the neuraxial unit as the efferent neurone and not as the reflex arc, and to regard as the primitive activity not the reflex but that rhythmic phenomenon of which there still remain examples in the acts of progression and respiration. On this basis it is possible to frame a hypothesis of the evolution of function in the nervous system on a more satisfactory foundation than that which is generally assumed when the reflex is regarded as the unit.

It must be clear that in the line of vertebrate evolution the first and most important activity is that of progression, for long before the presence of mechanisms for respiration became factors in the conservation of lines of descent a mechanism for changing the topographical relations of animal and environment played a part in that conservation.

Thus it is in the first place possible to figure an animal in which respiration took place as a direct interchange between cell and environment; and to picture it with a primitive locomotory mechanism which consists in an efferent neurone, the cell-body of which is directly exposed to the environment, and a primitive effector organ.

When the geographical relations between animal and environment remained too long unchanged there would occur a local asphyxia (in its widest sense—"starvation") of the efferent neurone—the primitive centre—and an activation of the effectors, even if incoördinate, would serve to drive the animal somewhere—anywhere—to a new region in which the deficiency of nutritive materials, and the surfeit of waste materials, would be compensated. In this primitive arrangement the discharge of the centre may be looked upon as prolonged in an "after-discharge" which lasts as long as the asphyxiation of the centres continues—unless indeed there occurs "fatigue" by the burning up of the molecules the katabolism of which subserves the activity of the centre. And the environmental stimulus may be regarded as the primitive "blood-stimulus."

The next stage in the evolution of the nervous system might be the primitive coördination of antagonistic effectors by means of the appearance of the process of mutual inhibition. It is possible to suppose that the primitive efferents in some lines of descent became able to inhibit efferent cells which activated muscles antagonistic to their own. When local asphyxiation then played upon the system the activity conditioned would not be the former incoördinate individual activity which was characterised by "after-discharge," but a coördinate rhythmic activity—the primitive progression.

As evolution of the type proceeded, and the primitive nervous system became enclosed, the local asphyxia of the environment no longer would directly affect the nerve centres and its place would be taken by the "blood-stimulus" similar to that which we may suppose to-day acts in even the highest forms. The general hunger of the animal would now set into activity its mechanism of progression by activation of linked and antagonistic centres (or "half-centres") and thus it would be driven into fresh regions of the environment.

But, still later, mechanisms of protection and attack and mechanisms for the perfection of progression became factors of importance in the conservation of lines of descent. These might arise with the appearance of the peripheral afferent mechanism. Neurones which conveyed a state of raised activity towards the enclosed centres might make their appearance and might condition the activation of the centres by producing a state similar to that evoked by the "blood-stimulus." At first these might activate the linked "half-centres" equally, just as the "blood-stimulus" may be supposed to do; but later they might condition a greater and finer selection, so that one "half-centre" alone might be activated.

Thus finally the peripheral reflex as we know it in the mammal might be produced as a modification of the primitive neural activity first seen in its coördinate form in the "beat" of rhythmic progression.

SUMMARY.

1. In this paper are described experiments which demonstrate that the phenomenon of "narcosis progression" in the cat may occur at a depth of narcosis at which the spinal reflexes are abolished, and at which even so great a flexion-producing stimulus as rapid division of the thoracic spinal cord is apparently without effect.

2. At this depth of narcosis the phenomenon of "rebound" also is abolished.

3. Previous experiments have demonstrated that rhythmic phenomena are conditioned during the balance of equal and opposite activities in the antagonistic centres (or "half-centres"); but as these centres when activated from a single side demonstrate arhythmic activity it has been pointed out that it is not justifiable to assume that the rhythm is conditioned in the individual cell-body or "half-

centre." The inference has been drawn that the rhythm is conditioned by properties of the mutual interconnexions of the "half-centres," and that it occurs during their equal activation.

4. But that this activation of the antagonistic linked "half-centres" is not necessarily of peripheral origin is shown by the present experiments.

5. It is therefore suggested that the equal and opposite activation of the linked antagonistic "half-centres" may be fundamentally conditioned by a stimulus equivalent to the "blood-stimulus" in respiration—although in normal circumstances this may be aided by the peripheral impulses. That asphyxia may affect the phenomenon of progression has already been shown.

6. But when the peripheral reflexes are abolished during the occurrence of "narcosis progression" there are still evident in that phenomenon many of the attributes of reciprocal innervation.

7. The inference is therefore drawn that a mechanism for that reciprocal innervation may exist between antagonistic efferent neurones (antagonistic "half-centres") without necessitating the presence of afferent neurones. Arising out of this conception a theory is given to account for the manner of the conditioning of rhythmic activity.

8. The experiments seem to show that the fundamental unit of activity in the nervous system is not that which we term the spinal reflex. They show the independence of the efferent neurone, and suggest that the functional unit is the activity of the independent efferent neurone; or rather, that it is the mutually conditioned activity of the linked antagonistic efferent neurones ("half-centres") which together form the "centre": and they also suggest that the primitive activity of the nervous system is seen in such rhythmic acts as progression and respiration.

9. In the light of this view it is suggested that the spinal reflex is of the nature of a distorted "beat" of the rhythmic progression phenomenon.

10. On the basis of the suggested functional independence of the efferent neurone or "half-centre" a theory of the manner of the evolution of function in the nervous system is sketched. It is supposed that at first the "half-centre" was directly exposed to the environment and that a state of "local asphyxia" then acted as the equivalent of the "blood-stimulus" in activating it and thus producing movements of the effector. Such rough movements might serve to change the immediate surroundings of the animal. Then the primitive coördination arose

in the appearance of a system of mutual inhibition between "half-centres" which innervated antagonistic effectors. At this stage rhythmic activity became possible, and with it a more efficient locomotion of the animal. With the enfolding of the nervous system the primitive environmental direct stimulus was replaced by the indirect "blood-stimulus"; and a peripheral afferent nerve mechanism, the function of which was to perfect the movements of progression and to give reflex movements of attack and defence, arose.

11. In other words, the general bearing of these experiments is to throw doubt upon the spinal reflex as the unit of function of the nervous system and to emphasise the possibility of a more fundamental unit of activity conditioned in independent "half-centres" by stimuli equivalent to the "blood-stimulus."

HYPERPNŒA AS A RESULT OF PAIN AND ETHER IN MAN.

By A. L. MEYER, M.D. (*University of Pennsylvania.*)

(*From the Physiological Laboratory, Oxford.*)

YANDELL HENDERSON has shown that "the condition of shock which results in man from extreme physical suffering is to a large extent caused by the reduction of the CO_2 content of the blood and tissues by the violent and prolonged hyperpnœa induced by stimulation of afferent nerves(1)." Haldane and Poulton(2) and others have observed unpleasant and even alarming symptoms arising from forced breathing. Henderson, in experiments carried out on sixty young men, found that forced breathing can produce symptoms bearing a strong resemblance to shock(3). In six dogs(4) the sciatic nerve was divided and an electrical stimulus applied to the central end. The hyperpnœa incident to the stimulation was continued for twenty minutes, when apnœa immediately followed. Three of the dogs died of cardiac failure after an apnœa of from 8–12 minutes. In another series of twenty dogs manipulation of the abdominal viscera was used as a means of inducing hyperpnœa, after an hour and a half fatal apnœa resulted in all those cases in which the irritation was discontinued.

Henderson has also given experimental evidence in support of the view that ether is capable of producing an hyperpnœa and a subsequent fatal apnœa. Four dogs(5) were subjected to about twenty minutes of ether-hyperpnœa. No morphin was used. Deep anæsthesia brought on a short apnœa with spontaneous recovery in three of the dogs. Light anæsthesia again threw the dogs into hyperpnœa and ultimately profound acapnia. Hence the conclusion that "ether-hyperpnœa is quite as effective as pain-hyperpnœa or excessive artificial respiration as a means of inducing a subsequent fatal apnœa vera."

The above conclusions were largely drawn from experiments upon animals. Furthermore, the measures employed in producing hyperpnœa were "vigorous and prolonged" and the mental element of pain was absent owing to the anæsthesia. In view of these considerations and also in view of the fact that hyperpnœa and acapnia have been definitely related with one of the most important symptom-complexes in medicine Dr Haldane suggested that it would be well to determine the effect of moderate pain and of the incipient stage of anæsthesia in man, with definite determinations of the volume of air breathed, the respiratory exchange, and the alveolar CO_2 pressure.

Pain-hyperpnœa.

In investigating the effect of moderate pain in man experimentally it was of course essential that the duration of the pain should be within control. The simple method of placing a clamp on the end of the little finger was adopted. The clamp was screwed down firmly enough to produce a blue-nail in two or three instances. The average pain was probably such as might be caused by a severe paronychia. In the first experiments the clamp remained on the finger for two minutes, in the later experiments, three minutes. The exact amount of hyperpnœa was determined by actually measuring the volume of expired air in rest and in pain. Two small Douglas' bags(6) were used,—one for the expired air in rest, the other for the expired air in pain. Having attached a long flexible tube, provided with an inspiratory and expiratory valve, to one of the bags I seated myself in a comfortable position and tried to secure the utmost relaxation. When the respiration and pulse became normal I breathed into the bag for 2 to 3 minutes. After a short interval the clamp was applied, the desired amount of pain produced, and the second bag filled in the same manner. The air was next sent through a meter to measure the exact quantity. While the air was passing a sample was taken in a sampling-tube attached to the rubber tubing between the bag and meter. This was analysed by the Haldane method. Having the composition of the air and the quantity expired it was possible to calculate the respiratory quotient and exchange. The results appear in Table I.

An inspection of these figures shows that pain even of moderate intensity and of short duration is capable of producing a material hyperpnœa. The quantity expired during hyperpnœa incident to pain is from 10 to 56 % higher than the quantity expired during rest. The respiratory quotient likewise increases noticeably—from 4 to 25 %. The variation in the results must be attributed partly to the extreme difficulty of securing pain of uniform intensity. The adjustment of the clamp varied somewhat, and besides the subject would tend to adapt himself to pain of a given intensity after successive applications. The point to be emphasized however is that the degree of pain actually suffered did produce hyperpnœa.

TABLE I.

Time = 2 mins.

Exp. air				Quant. expired in litres		Resp. quotient		Resp. exchange			
CO$_2$		O$_2$						CO$_2$ given off		O$_2$ absorbed	
rest	pain	rest	pain	rest	pain	rest	pain	rest	pain	rest	pain
3·52	3·64	16·84	17·14	13·0	17·0	·82	·94	·453	·613	·551	·652
3·71	3·69	16·86	17·25	14·7	23·0	·88	·99	·540	·841	·612	·846
3·62	3·60	16·56	17·60	15·3	20·1	·78	·89	·549	·717	·699	·808
Time = 3 mins.											
3·73	3·81	16·88	16·95	23·2	28·0	·89	·93	·858	1·058	9·60	1·128
3·49	3·60	17·00	17·10	22·5	26·0	·85	·91	·778	·928	·991	1·011
3·42	3·48	17·08	17·46	20·5	26·1	·85	·99	·705	·900	·825	·905
3·35	3·36	16·94	17·57	19·9	27·65	·79	·99	·660	·920	·827	·931
3·58	3·52	16·98	17·17	22·4	24·7	·85	·91	·784	·862	·911	·946
3·31	3·40	17·14	17·52	20·1	26·5	·83	·98	·659	·893	·787	·906
3·39	3·67	16·99	17·05	19·9	24·5	·82	·92	·668	·891	·813	·965

According to the results of the two minute experiments the normal elimination of CO$_2$ was about 0·257 litre per minute. During pain the average elimination of CO$_2$ rose to 0·362 litre per minute. This means that 105 c.c. more CO$_2$ were given off during pain than during rest. In the same experiments the average consumption of O$_2$ under normal conditions was 0·316 litre per minute and during pain 0·384 litre, an increase of only 0·074 litre. In the three minute experiments the normal output of CO$_2$ amounted to 0·238 litre per minute, whereas during pain this output increased to 0·307 litre. The normal oxygen consumption per minute was 0·291 litre, increasing to 0·323 litre during pain. Hence in both instances more CO$_2$ is being lost than can be accounted for by the increased absorption of oxygen. This indicates that under the conditions there was a distinct tendency toward an acapnic condition.

It was therefore of interest to examine the alveolar air during pain and determine the effects on the alveolar CO$_2$. The clamp was allowed to remain on the finger for one minute, when a sample of the alveolar air was taken by the method of Haldane and Priestley. This was then analysed and compared with the percentage composition of the air previous to the hyperpnœa. The analyses follow (Table II).

These figures reveal a marked drop in the alveolar CO$_2$ during pain—the difference between the normal percentage and the percentage during pain amounting to an average of 0·72 °/$_0$.

TABLE II.

Composition of alveolar air			
CO_2		O_2	
rest	pain	rest	pain
5·01	4·64	15·20	16·38
5·31	4·61	14·32	16·53
5·34	4·67	14·38	16·46
5·31	4·31	14·91	17·10
5·54	4·88	14·22	16·25
5·37	4·62	14·67	16·06
5·61	4·69	14·23	16·05

It will also be noticed that whereas the average fall in the alveolar CO_2 is 0·72 °/₀, the average fall in the oxygen deficiency is 1·84 °/₀. This is due to the hyperpnœa. Were it not for the fact that CO_2 was passing into the alveoli from the blood in an abnormal proportion, the alveolar percentage of CO_2 would have been much less.

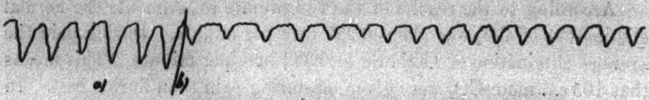


Fig. 1. (*a*) Hyperpnœa of pain (1 min.). (*b*) Cessation of pain.

In view of the hyperpnœa and the diminution in the alveolar CO_2, a stethographic record (Fig. 1) was obtained during and after the pain in order to observe the character of the respiratory movements. It will be seen that during recovery the respirations were shallow, and the intervals between them were distinctly prolonged. While there is no complete apnœa yet the prolongation of the intervals points distinctly to the reduction in the CO_2.

Ether-hyperpnœa.

For the purpose of determining the effect of the incipient stage of etherization on man, a mixture of ether and air was made to pass through a bottle of 2460 c.c. capacity. The bottle was connected by flexible tubing with a dry meter. An inspiratory and expiratory valve was introduced between the bottle and meter. The mixture could in this way be made to pass through the bottle at each inspiration, and through the meter at each expiration. After first of all ascertaining the volume expired under normal conditions the ether mixture was inhaled and at the same time the volume of air expired, determined. The bottle was then disconnected and the percentage of ether in the mixture estimated. The principle of the estimation consisted in weighing the ether by Waller's method(7). In the first attempts to produce an hyperpnœa with ether I failed. This was due to the very weak mixture employed. As the strength of the mixture was increased hyperpnœa resulted. It was also observed that when the experiment was repeated several times in close succession with the same percentage of ether, the hyperpnœa became less and less. It was therefore necessary to perform but one or two experiments at a time.

A glance at Table III will show that a mixture of 1·8 °/₀ failed to give me any hyperpnœa. A mixture of approximately 5 °/₀ was the most effective, whereas a mixture of 10·3 °/₀ again failed to induce hyperpnœa and actually reduced the volume of air expired below the normal. In no instance is the response of my respiratory centre very great. It was found that in Dr Haldane's case the hyperpnœa was far more pronounced, even with comparatively weak concentrations. On one occasion a mixture of 3·3 °/₀ produced an increase of 92 °/₀ in the air respired; on another occasion a mixture of 2·8 °/₀ produced an increase of 66 °/₀. Persons probably vary considerably in the hyperpnœa which ether produces.

TABLE III.

Quantity expired normally in 1 min. (cu. ft.)	Quantity expired under ether in 1 min. (cu. ft.)	°/₀ increase in quantity expired	°/₀ ether in mixture
0·220	0·220	0	1·8
0·275	0·330	20	2·7
0·210	0·260	24	2·8
0·235	0·305	30	3·5
0·228	0·305	34	4·0
0·210	0·290	38	4·9
0·210	0·280	33	4·9
0·230	0·310	35	5·1
0·210	0·270	29	6·0
0·240	0·310	29	6·2
0·210	0·265	26	7·4
0·210	0·265	24	8·7
0·210	0·200	−4·7	10·3
0·205	0·190	−7·3	10·6

CONCLUSIONS.

I. Pain of moderate intensity and of short duration (2 to 3 mins.) produces hyperpnœa that can be easily measured. The alveolar CO_2 is diminished and the respiratory quotient increased.

II. Ether in suitable low concentration produces hyperpnœa in one minute. The amount of hyperpnœa varies in different persons; and with higher concentrations the breathing is diminished.

REFERENCES.

(1) **Yandell Henderson.** Amer. Journ. Physiol. XXI. p. 130.
(2) **Haldane and Poulton.** This Journal, XXXVII. p. 390. 1908.
(3) **Yandell Henderson.** Amer. Journ. Physiol. XXV. p. 315.
(4) Ibid. p. 394.
(5) Ibid. XXVI. p. 279.
(6) **Douglas.** Proc. Physiol. Soc. p. xvii. 1911 (this Journal, XLII.).
(7) **Waller.** Ibid. p. vii. 1903 (this Journal, XXX.).

THE RELATION BETWEEN THE SIZE OF THE PROPAGATED DISTURBANCE AND THE RATE OF CONDUCTION IN NERVE. BY E. D. ADRIAN, *Fellow of Trinity College, Cambridge.*

(*From the Physiological Laboratory, Cambridge.*)

THE experiments described in this paper were made to determine the relation between the size of the propagated disturbance in nerve and the rate at which it is conducted. If all other conditions remain constant, is a small disturbance conducted at the same rate as a large one, or is it conducted more rapidly or more slowly? In other words, is there any relation between the intensity of the disturbance at any point and the rate at which it spreads to adjacent points on the nerve fibre? The answer to this point is of some importance, for it is clear that it must be taken into account by any theory which professes to give a complete explanation of nervous conduction.

It is generally admitted that the rate of conduction in normal nerve does not depend on the strength of the stimulus which sets the propagated disturbance in motion. This would settle the question, if it could be shown that the size of the disturbance depended on the strength of the stimulus. However it is highly probable that the relation between stimulus and disturbance is an all-or-none relation[1], and therefore some other test must be applied. The most direct way of altering the size of a propagated disturbance, without altering any other conditions at the same time, consists in allowing the disturbance to pass through a region of decrement. In such a region the disturbance becomes smaller and smaller as it advances, but the condition of the tissue is the same at all points in the affected area. Thus, if it were found that the disturbance was conducted more and more slowly as it passed down the nerve, there could be no doubt that the reduced rate of conduction was due to the reduced size of the disturbance and to nothing else. At first sight it seems very likely that the rate of conduction is reduced in this way in a region of decrement. Certainly all conditions which lead to a progressive reduction in the size of the disturbance (injury, lack of oxygen or of necessary ions, narcotics etc.) have been found to lead also to a slowed rate of conduction, and it is not unreasonable to suppose that the slowing is due directly to the reduced size of the disturbance. However it is quite possible that the rate is uniformly slow at all points in the affected area, and the point cannot be decided without actual measurements of the rate of conduction at different points in the region of decrement.

---

[1] For a discussion of this point see Adrian, This *Journal*, XLVII. p. 460. 1914.

Measurements of the rate of conduction in a region of decrement were made three years ago by Koike[1]. The experiments were carried out on the sciatic of the frog and the olfactory nerve of the pike. The nerve was narcotised with ether, and the rate of conduction was determined by measuring the time which elapsed between the stimulus and the beginning of the electric response at different distances from the stimulating electrodes. Koike found that the rate was exactly the same at all points within the region of decrement, although it was considerably slower than the rate in normal nerve. He concluded that the rate of conduction did not depend on the size of the disturbance.

Koike's work has been criticised by Fröhlich[2]. He considers that Koike's experiments were too few and too inaccurate to justify his conclusions. Of these experiments four were made on the sciatic and one on the olfactory nerve. The latter was the most accurate, as the rate of conduction in the olfactory nerve is very slow, but the nerve is also very short, and the measurement of the rate of conduction at different points is by no means easy. Koike's measurements of the string galvanometer records agree well enough with one another and, allowing for the personal equation, with the measurements of an independent observer, but Fröhlich finds a greater variation when he measures the records himself. This criticism applies much more forcibly to the experiments on the frog's sciatic, where the error is admittedly large. However, in the absence of any evidence to the contrary these results do lend support to the view that the rate of conduction does not vary with the size of the disturbance. According to Fröhlich, evidence to the contrary is to be found in Koike's own records. He points out that in some of these records the time taken by the electric response to reach its maximum value increases as the disturbance passes down the region of decrement. Thus the smaller

[1] *Ztsch. f. Biol.* LV. p. 311. 1911.

[2] *Ztsch. f. allgm. Physiol.* XIV. p. 55. 1913.

the propagated disturbance, the slower is the rate of development of the response. He considers that a change in the rate of development of the electric response must imply a corresponding change in the rate of conduction. Consequently the conduction must become slower and slower as the size of the disturbance is reduced. In objection to this it might be urged that a slow rate of development of the response of the whole nerve trunk does not necessarily imply a slow development of the response in each nerve fibre. It might well be due to a difference in the rate of conduction of different fibres, brought about by unequal narcotisation. If such a difference were set up, the time taken by the electric response of the nerve trunk to reach its maximum would increase, although the response of each fibre remained unaltered. Furthermore this slowing of the nerve trunk response would increase with the length of nerve through which the disturbance passed, for the difference in the time of arrival of the disturbance in the slow and rapid fibres would become greater and greater as the length of nerve increased. Fröhlich considers this objection and concludes that it is untenable. He argues that, if the increased length of the response were due to differences in the time of arrival of the disturbance in different fibres, the response of the nerve trunk would still remain abnormally long after the disturbances had passed out of the region of decrement into normal tissue. Fröhlich carried out some experiments to test this point. The two sciatic nerves of a frog passed through a 20 mm. narcotising chamber, and electrodes were arranged to record the monophasic response at a point halfway through the chamber and at another point outside the chamber on the side removed from the stimulating electrodes. Eight successful experiments were made, and in all of them the electric response reached its maximum more slowly in the narcotised region than it did in the normal region beyond.

Fröhlich concludes that the prolonged nerve trunk response is due to a prolonged response in each nerve fibre, and that Koike's records are not to be explained by the assumption that different fibres may conduct at different rates. The first part of this conclusion is clear enough; the experiments show that the response may be prolonged in each fibre and that it regains its normal time relations on emerging into normal tissue. This is not surprising, for it is known that the rate of conduction is slower in the affected area than it is in the region beyond. It is clear too that all the fibres were conducting at the same rate. However this does not dispose of the question, for in these particular experiments there is nothing to show that the rate of development of the electric response became slower and slower as the disturbance passed down the nerve; it might well have been uniformly slow at all points in the affected area. Nor do **Fröhlich**'s results prove that an unequal narcotisation of different fibres is an impossibility; that it could not have occurred in some of **Koike**'s experiments and accounted for the progressive change in the rate of development of the disturbance. Indeed it is difficult to see how such inequalities could have been avoided, since **Koike** used four sciatic nerves placed side by side, and there is always some slight difference in the rate of narcotisation of the nerves of different frogs. To prove **Fröhlich**'s point it would be necessary to show in one and the same preparation that the response became more and more prolonged as it passed down the narcotised region, and also that it was not at all prolonged after it had emerged into normal nerve. This would show that the effect, when it did exist, was not due to different rates of conduction in different fibres.

Even if **Fröhlich** had given a rigid proof that the electric response in each fibre was developed more and more slowly as the propagated disturbance was reduced in size, it would still be unnecessary to accept the conclusion that the rate of conduction decreased in the same way. The passage of an electric response down the nerve is often described as the passage of a 'wave of negativity.' Using this description we may say that the rate of development of the response at any point may be taken as a measure of the rate of conduction, provided that the wave length of the response remains constant. But we have no right to assume that the wave length will remain constant when the size of the disturbance is reduced. Therefore, in the present experiment where the size of the disturbance is changing, the rate of development of the response, taken apart from any other evidence, cannot be used as a true measure of the rate of conduction in the region of decrement.

Thus the question is still unsettled, if we consider that **Koike**'s measurements of the rate of conduction are not accurate enough. In his method the chief source of error lies in the interpretation of the string galvanometer records. It is very difficult to determine the exact moment at which the electric response begins, and difficult to determine the probable error which is introduced into these measurements, as the responses are not all of the same size. This uncertainty may be avoided by using the method recently introduced by **Keith Lucas**[1] for measuring the rate of conduction in nerve.

---

[1] This *Journal*, XLVI. p. 499. 1913.

Lucas' method consists essentially in determining the time at which different points on the nerve reach a definite stage of recovery after the passage of a propagated disturbance. When the nerve of a nerve-muscle preparation is excited by two stimuli separated by a very short time interval, the second stimulus may succeed in calling up a summated contraction in the muscle, or it may have no apparent effect. The least interval which will suffice for the production of a summated contraction (the 'interval for muscular summation') is the least interval at which the nerve has recovered to such an extent that the second disturbance is large enough to affect the muscle. Consequently by measuring this interval we can find the time taken by the nerve to reach a certain stage of recovery after the first disturbance. If both stimuli are applied to the same point on the nerve, the whole of the interval will be occupied in the processes of recovery. On the other hand, if the first stimulus is sent in higher up the nerve than the second, the interval for muscular summation will be made up of the time taken in recovery plus that taken in the conduction of the first disturbance from the seat of the first stimulus to that of the second. Thus the time taken in conduction from a point x to a point y is simply the interval for summation with the first stimulus at x and the second at y minus the interval with both stimuli at y.

There is usually no difficulty in measuring the interval for muscular summation correct to ·00008 sec., and in any case the greatest possible error is always known, since it consists in the difference between the least interval at which the two stimuli were found to give a summated contraction and the greatest interval at which they gave a single twitch only.

The details of the method are described in the following section.

Method of measurement. For the measurement of the rate of conduction in narcotised tissue it is of the greatest importance that the narcosis should proceed slowly and uniformly at all points on the nerve, and also that there should be no danger of current spread from the stimulating electrodes. Both these conditions are secured by **Lucas'** plan of narcotising with a slow stream of alcohol dissolved in Ringer's fluid and stimulating by means of fluid electrodes in which the lines of current flow are suddenly constricted round the nerve. The arrangement of these electrodes has been described in detail elsewhere[1]. Briefly, the nerve passes through a series of chambers connected by narrow slots and filled with Ringer's fluid, or a solution of alcohol in Ringer's fluid.

---

[1] This *Journal*, XLVI. p. 480. 1913, and XLVII. p. 465. 1914.

The stimulating current passes in the fluid from one chamber to the next, and the lines of current flow are suddenly constricted in the neighbourhood of the slot. Fig. 1 shows a plan of one of the troughs used in the present experiments. The trough is cut out of ebonite, and the chamber *M* contains the gastrocnemius pinned to the cork floor and covered with Ringer's fluid. The nerve passes through a series of seven holes 6·35 mm. in diameter, and connected by slots 2·5 mm. deep, 1·5 wide, and 1·5 long[1]. Glass tubes T_1 and T_2, lead from the two end holes and serve for the entrance and exit of the perfusing fluid. The temperature is recorded by a thermometer fixed with its bulb in the middle chamber. The stimulating current is led into each chamber (including the muscle chamber) by small flat coils of platinum wire *P*, soldered to copper wires which pass to the outside of the trough.

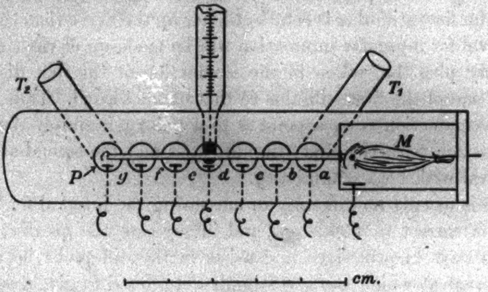


Fig. 1.

When the nerve is in position, the slot *a* leading to the muscle chamber is plugged with vaseline, and the whole system is filled with Ringer's fluid and covered with a glass plate smeared with vaseline. The nerve may now be stimulated at any of the slots (*a, b, c, d, e, f, g*) by connecting two adjacent platinum rings with the terminals of an induction coil. Owing to the nature of the electrodes, the danger of current spread is reduced to a minimum; indeed the current may be increased to 23 times its threshold value without affecting the nerve 4 mm. away from the slot. The connections are arranged so that one or both of two coreless induction coils can be connected with any one slot electrode. Break shocks are used, and the coils are so arranged that the cathode at break is always on the muscle side of the slot. The circuits are broken by a Lucas pendulum.

---

[1] Three chambers were used, and the dimensions of two of them approximate closely with those given in the figure. In the third (used in Exp. 6) the holes were 9·5 mm. in diameter.

At the beginning of each experiment the rate of conduction is determined in the fresh nerve. During this determination the nerve is perfused with a slow stream of Ringer's fluid from a Mariotte bottle. When the nerve is to be narcotised, alcohol dissolved in Ringer's fluid is substituted for the plain Ringer, and this is perfused until the end of the experiment.

The rate of conduction in the fresh preparation is measured as follows. The strength of the stimuli is first adjusted so that the stimulus from the first coil is about twice and that from the second about six times[1] as strong as the threshold stimulus. Then the interval for muscular summation is determined, first of all with both stimuli at electrode *a* (nearest the muscle), and then the second stimulus at *a* and the first at all the other electrodes in turn. The measurements are repeated to make certain that the preparation is in a steady condition, and if the two series agree, the rate of conduction may be calculated at once. The method of calculation may be seen from the example given below in Table I. This experiment was made to determine the effect of leaving the preparation for some hours set up in the chamber and perfused with Ringer's fluid. The rate of conduction was determined ½ hour after the preparation was set up, and again after six hours in the chamber. The following measurements were made when the preparation was fresh.

These measurements are plotted as shaded rectangles in Fig. 2. Ordinates represent the conduction time in seconds, and abscissae distances in mm. The possible error in each measurement is given by the size of the rectangle (it is assumed that the maximum error in the measurement of length is always 1 mm.). Evidently the points all lie on a straight line, as they should do if the rate of conduction were the same in every part of the nerve. The rate of conduction over 49 mm. is 31·5 ± 2 metres per sec. Thus there is an error of ± 6·3 %. This error depends on the fact that the intervals for muscular summation cannot be measured conveniently to within less than ·00008 sec. Fortunately the percentage error due to this will decrease as the rate of conduction decreases, for when this happens the conduction time will be longer, but the error in its measurement will still be the same, viz. ·00016 sec.

---

[1] When the second stimulus is as strong as this, a small alteration in its strength will not affect the length of the interval for muscular summation. This avoids the necessity for a third coil always in parallel with the second (cf. Lucas, This *Journal*, XLVI. p. 502. 1913).

TABLE I. Exp. 1. *Nerve perfused with Ringer's fluid for 15 minutes. Temperature* 16·1° C.

	Interval for muscular summation [1]	Time spent in conduction [2]	Distance between seat of first and second stimulus
Second stim. at *a*,			
First stim. at *a*	·00184 sec.	0 sec.	0 mm.
	·00176		
First stim. at *b*	·00216	·00040	
	·00208	·00024	9·0 ± ·5
First stim. at *c*	·00240	·00064	
	·00232	·00048	17·5 ± ·5
First stim. at *d*	·00264	·00088	
	·00256	·00072	25·0 ± ·5
First stim. at *e*	·00288	·00112	
	·00280	·00096	33·5 ± ·5
First stim. at *f*	·00320	·00144	
	·00312	·00128	41·0 ± ·5
First stim. at *g*	·00340	·00164	
	·00332	·00148	49·0 ± ·5

Conduction time over 49 ± ·5 mm. lies between ·00164 and ·00148 seconds.

Therefore the rate of conduction over the nerve from *a* to *g* lies between 33·5 and 29·5 metres per second.

[1] The numbers in this column give the nearest intervals at which it was clear that the two stimuli gave (1) a single twitch, and (2) a well-marked summated contraction. Thus both stimuli at *a* gave summation at an interval of ·00184 sec. and no summation at an interval of ·00176 sec.

[2] This column gives the maximum and minimum values for the conduction time over different lengths of nerve. The conduction time from *a* to *b* is equal to the interval for muscular summation with the first stimulus at *b* and the second at *a*, minus that with both at *a*. Thus the maximum value for the conduction time over 9 mm. equals ·00216 − ·00176 (*i.e.* ·00040 sec.), and the minimum value equals ·00208 − ·00184 (*i.e.* ·00024 sec.).

The white rectangles show the measurements made after the preparation had been six hours in the chamber. They give a mean rate of conduction of 24·7 metres per sec., the temperature having risen from 16·1 to 16·4° C. during the interval between the two determinations. This result is useful in showing that we need not fear any considerable change in the rate of conduction when the preparation is left for an hour under normal conditions. Therefore if a pronounced alteration is met with in a narcotising experiment, it is safe to assume that this alteration is due almost entirely to the experimental change of conditions. Another point of interest is the fact that the plug of vaseline between the electrodes *a* and *b* does not injure the nerve to the extent of altering its rate of conduction more rapidly than it is altered by contact with the Ringer's fluid.

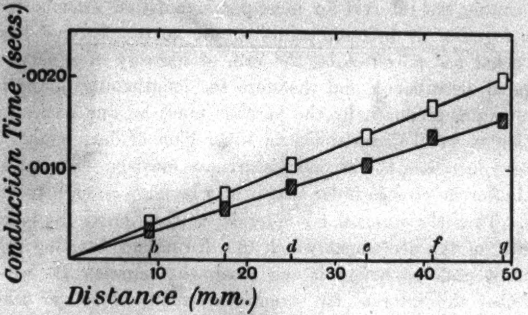


Fig. 2.

The following section deals with the experiments in which the rate of conduction was determined in narcotised tissue.

Rate of conduction in narcotised nerve.

The following experiments were made to compare the rates of conduction at different points in a nerve conducting with a decrement. It is obvious that this cannot be attempted unless all the measurements are made with the nerve in approximately the same condition. Fortunately the method of perfusion with alcohol solution satisfies this condition. By careful adjustment of the strength of the solution and the rate of perfusion it is possible to bring the nerve into a condition in which the degree of narcosis remains constant for half an hour or even longer. The correct strength of the solution varies with the temperature and the condition of the nerve; in summer at temperatures of about 20° C. the right strength is usually 2·5 to 3 %, in winter at 14° to 15° C. it is about 5 %. In many cases the constant state is never reached, and the nerve is narcotised too rapidly or not at all; however, if precautions are taken to keep the temperature of the room constant, the steady condition may be attained in at least two-thirds of the total number of experiments made.

A few preliminary experiments showed that a very weak alcohol solution might reduce the rate of conduction to some extent without causing the nerve to conduct with a decrement. Obviously the comparison of the rate of conduction at different points can have little bearing on the question in hand, unless it is quite certain that the disturbance becomes smaller as it passes down the nerve. However, the extent of the decrement suffered by the disturbance may be determined by measuring the interval for muscular summation with both stimuli sent in together at different points on the nerve. Lucas[1] has shown that alcohol has no effect on the rate of recovery of a nerve from a propagated disturbance, and therefore the lengthening of the interval for summation produced by the narcosis must be due entirely to the extinction of small disturbances in the region of decrement. As the decrement increases, the second disturbance must be set up at a longer interval after the first in order that it may be large enough to reach the muscle. Thus the interval for muscular summation at any point gives a measure of the decrement which the disturbance starting from that point must undergo before it can reach the muscle. So we should expect that the interval for summation would be longer when the stimuli were sent in at a point further removed from the muscle, and if the nerve is narcotised uniformly, the interval should increase quite regularly as the distance to the muscle is increased. For this reason measurements of the interval with both stimuli together at different points show not only whether the nerve is conducting with a decrement, but also whether this decrement is uniform. Such measurements were made in most of the experiments to be described. In the remainder there was no doubt as to the condition of the nerve, since conduction from the highest electrode to the muscle had failed completely before the measurements were taken.

The rate of conduction in narcotised nerve is determined in much the same way as in Exp. 1. The second stimulus is arranged to fall at the electrode *a* (at the peripheral end of the nerve), and the first stimulus at any of the electrodes from *a* to *g*. The interval for muscular summation is measured first of all with the first stimulus at *a*, then at *b*, *c*, *d*, *e*, *f*, and *g*. The series is then repeated in the reverse order with the first stimulus at *g*, *f*, *e*, and so on. This does away with the error which might be introduced if the state of the nerve were changing rapidly during the first series of measurements. After this the interval for muscular summation is determined with both stimuli together at *g*, *f*, *e*, etc. to make sure that the nerve is conducting with a decrement.

Fifteen successful experiments were made; in twelve of these the nerve was in a steady condition during the measurements, and in the other three the rate of change was so small that the conduction times had not altered by more than ·00024 sec. when the measurements were repeated in the reverse order. In all these experiments the rate of conduction was reduced considerably, and the disturbance was conducted with a decrement.

---

[1] This *Journal*, XLVI. p. 470. 1913.

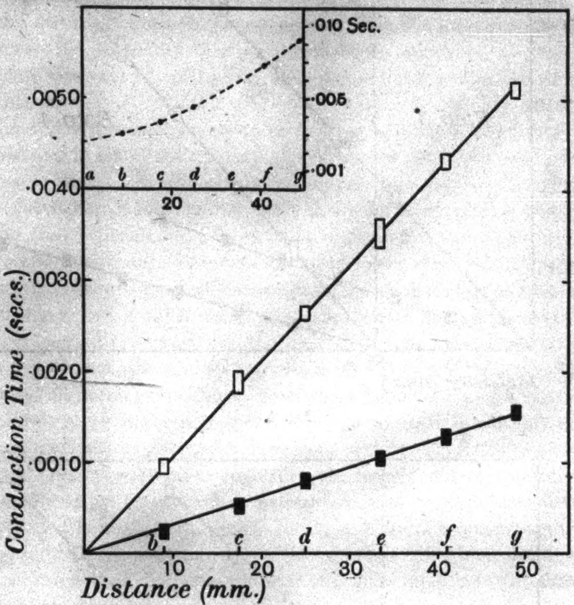


Fig. 3.

Figs. 3, 4, and 5 are constructed from five of the experiments in which the conditions remained constant (Exps. 2–6). The black rectangles denote measurements made on the fresh nerve, and the white rectangles those made during narcosis. The latter show the greatest and least possible values for the conduction time found in the course of the two independent determinations at each point. The small inset in Fig. 3 shows the interval for muscular summation with both stimuli together at the different electrodes. The curve rises steeply and regularly, and therefore the nerve must be narcotised throughout its whole length and the disturbance must be conducted with a decrement. Similar curves were obtained in the other experiments.

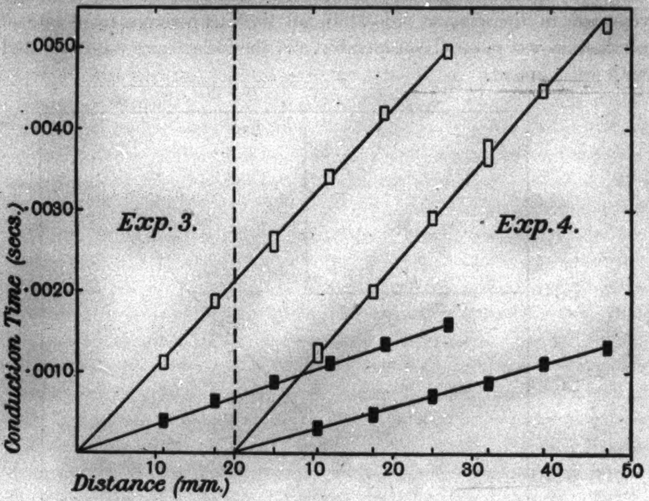


Fig. 4.

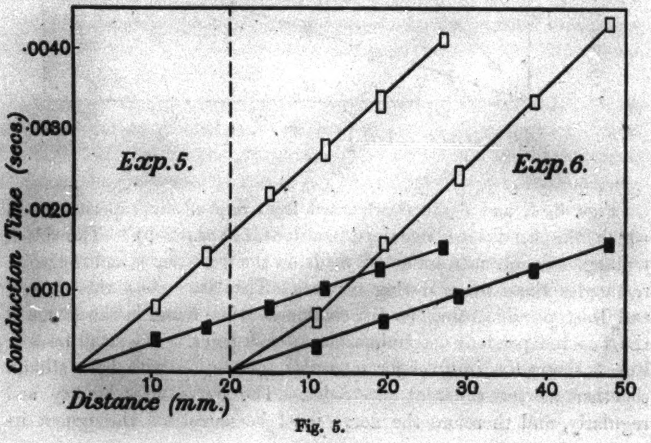


Fig. 5.

If we leave out of account for the moment the rate of conduction between the electrodes *a* and *b*, the result is clear. The rate of conduction is reduced to much less than half its original value, but the time spent in conduction varies directly with the distance travelled by the disturbance, and therefore the rate of conduction is the same at all points on the nerve. The disturbance set up at *g* or *f* will be very much reduced by the time it reaches *b*; it will certainly be smaller than the disturbance which was set up at *c*. Yet the rate of conduction is always the same whether the disturbance starts at *c*, *f*, or *g*. Evidently the rate of conduction does not depend on the size of the disturbance.

This conclusion might seem to be opposed by Exps. 5 and 6 (Fig. 5). In these and in six of the remaining experiments the rate of conduction between *b* and *a* was appreciably faster than that between *a* and any other electrode. However, this effect is not at all surprising when we consider that between *a* and *b* the nerve changes from the narcotised to the normal condition. The stimulus at *a* takes effect on the muscle side of the vaseline plug which separates the alcohol solution from the plain Ringer, and it takes effect at a point where the condition of the nerve is perfectly normal, for the excitability at *a* does not change however deeply the nerve may be narcotised. Thus the transition from narcotised to normal must occur somewhere between *a* and *b*, and so a part of the nerve between *a* and *b* will be narcotised less deeply than the rest, and will be able to conduct more rapidly. The length of this region of transition seems to depend on the length of nerve covered by the vaseline plug, for the effect may be increased or diminished at will by altering the size of the plug. In Exps. 2 and 3 the plug was very narrow and the effect is scarcely visible. In Exp. 6 the plug was 5 mm. long and the rate of conduction is obviously more rapid between *a* and *b* than it is anywhere else.

When this effect is taken into account the experiments agree in showing that the rate of conduction is the same at every point in the narcotised area whatever the size of the propagated disturbance may be. The degree of accuracy of this result may be seen from Table II which gives the actual figures for Exp. 2 (Fig. 3).

It will be seen that the mean values for the rate of conduction in the narcotised nerve lie between 9·35 and 9·55 metres per sec. whatever the length of nerve over which the rate is measured. When the nerve was fresh the mean value was 31·6 metres per sec. Naturally the possible error is greatest when the length of nerve is shortest, but it is

TABLE II. Exp. 2. *Nerve perfused with 5 % alcohol solution for 31 minutes. Temperature* 16·2° C.

	Interval for muscular summation (seconds)			Conduction time (secs.)	Distance (mm.)
	1st Series	2nd Series	Extremes		
2nd stim. at *a*					
1st stim. at *a*	·00264	·00264	·00264	0	0
	·00256	·00256	·00256		
1st stim. at *b*	·00360	·00360	·00360	·00104	9·0 ± ·5
	·00352	·00352	·00352	·00088	
1st stim. at *c*	·00456	·00448	·00456	·00200	17·5 ± ·5
	·00448	·00440	·00440	·00176	
1st stim. at *d*	·00528	·00528	·00528	·00272	25·0 ± ·5
	·00520	·00520	·00520	·00256	
1st stim. at *e*	·00624	·00608	·00624	·00368	33·5 ± ·5
	·00616	·00600	·00600	·00336	
1st stim. at *f*	·00696	·00696	·00696	·00440	41·0 ± ·5
	·00688	·00688	·00688	·00424	
1st stim. at *g*	·00776	·00776	·00776	·00520	49·0 ± ·5
	·00768	·00768	·00768	·00504	

Therefore rate of conduction between

	b and *a*	*c* and *a*	*d* and *a*	*e* and *a*	*f* and *a*	*g* and *a*	
Maximum	10·7	10·2	10·0	10·1	9·8	9·8	metres per sec.
Minimum	8·2	8·5	9·0	8·9	9·2	9·3	
Mean	9·45	9·35	9·5	9·5	9·5	9·55	

(The intervals for muscular summation with both stimuli together were

at *a*	at *b*	at *c*	at *d*	at *e*	at *f*	at *g*
·00264	·00320	·00384	·00472	—	·00696	·00840
·00256	·00304	·00360	·00432	—	·00664	·00800

N.B. These measurements are not made with any great degree of accuracy, as their only purpose is to show that the nerve is conducting with a decrement, and that it is narcotised uniformly.)

clear that the rate shows no obvious tendency either to decrease or to increase as the size of the propagated disturbance is reduced. This is shown most clearly in the figure. The greatest possible error is given by the size of the rectangles, and it would be extremely difficult to join the rectangles by anything but a straight line. If they lie on a curve, there is nothing to show whether it is concave upwards or downwards, and its curvature must be very small indeed.

The temperatures and mean rates of conduction in Exps. 2 to 6 are given in Table III. In these experiments the rate for the narcotised nerve varies between 8 and 12 metres per sec. As a rule the rate was

7–9 metres per sec. at 17°C. when the narcosis was deep enough to extinguish a disturbance in 40 mm. of nerve. When the disturbance was extinguished in 25 mm. the rate was often as slow as 4 to 5 metres per sec.

TABLE III.

Exp.	Temperature	Mean rate of conduction (metres per sec.) Fresh	Narcotised
2	16·2° C.	31·6	9·5
3	17·0	29·4	9·45
4	20·9	35·6	8·9
5	16·7	30·0	11·4
6	17·3	30·1	11·1

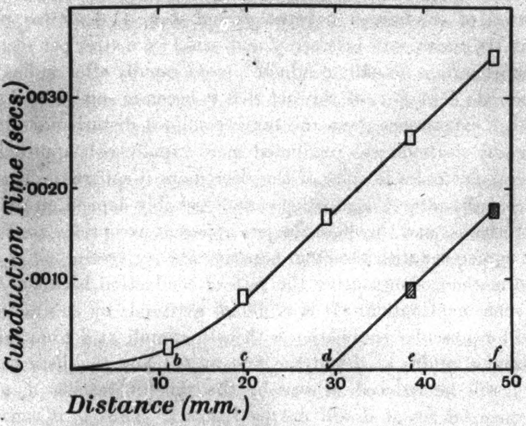


Fig. 6.

In fifteen experiments the rate of conduction appeared to be quite independent of the size of the propagated disturbance, but to this result there were two apparent exceptions (Exps. 7 and 8). In both of these the nerve was in a steady condition of narcosis, but the rate of conduction was not uniform. One of these experiments (Exp. 7) is shown in Fig. 6. The white rectangles give the conduction times from the different electrodes to *a* measured in the usual way. The rate from *b* to *a* is faster than the rate anywhere else, but this is not all, for the rate from *c* to *b* is clearly faster than that from *d* to *c*. It is true that from *f* to *c* the rate is constant, but the curve joining the different points is decidedly concave between *c* and *a*. Is this due simply to an unequal narcotisation of the nerve, or does it show that the rate of conduction sometimes depends on the size of the disturbance? In both of these experiments there can be little doubt that the former explanation is correct and that the effect is due to the nerve between *a* and *c* being less deeply narcotised than that higher up. This is shown by the lower curve in Fig. 6. The shaded rectangles are measurements of the time taken in the conduction to *d* of a disturbance set up at *f*. If the nerve is narcotised uniformly, this time should be equal to that taken in the conduction to *a* of a disturbance set up at *c*, since both will undergo the same decrement. As a matter of fact the conduction time from *f* to *d* is very much longer than that from *c* to *a*; ·0017 sec. between *f* and *d* as against ·0008 sec. between *c* and *a*. The mean rate of conduction between *f* and *d* is 11·3 metres per sec., whereas the mean rate between *f* and *a* is 14·4 metres per sec. Thus the disturbance is actually conducted more rapidly after it has passed the electrode *d* in spite of the fact that it becomes smaller and smaller. The other experiment gave the same result; a disturbance set up at the highest electrode was conducted more rapidly as it approached the peripheral electrodes in spite of the decrement it suffered. This result shows that the rate of conduction cannot possibly depend on the size of the disturbance, and therefore the two apparent exceptions are really in perfect agreement with the other experiments.

The method of measuring the rate of conduction between *f* and *d* needs some explanation. It was found, as usual, by subtracting the interval for muscular summation with both stimuli at *d* from that with the second stimulus at *d* and the first at *f*. Now the disturbance set up at *f* will be reduced in size by the time it reaches *d*, and the disturbance set up at *d* will not be reduced. Thus it is conceivable that the nerve at *d* might take longer to recover from the disturbance starting at *d* than from that starting at *f*. No allowance has been made for this in calculating the rate of conduction, for it is assumed that the rate of recovery of the nerve is the same for both disturbances. Fortunately this does not affect the result in the least. If the nerve takes a longer time to recover from the unreduced disturbance set up at *d*, the conduction time between *f* and *d* may appear shorter than it is in reality, but it cannot possibly appear longer. This may be seen from the fact that if the difference in recovery times were very great, the apparent conduction time between *f* and *d* might be nothing at all, or even a minus quantity, since the true conduction time from *f* to *d* plus the recovery time of the reduced disturbance might be equal to or even less than the recovery time of the unreduced disturbance set up at *d*. Thus there is no danger that the conduction time between *f* and *d* will appear longer than it is in reality, and therefore the conclusion arrived at with regard to the two experiments holds good in spite of the uncertainty regarding the time taken to recover from disturbances of different sizes.

In a number of experiments the state of the nerve changed rapidly during the measurements, making it impossible to compare the rates of conduction over different lengths without introducing a very large error. In none of these was there anything to suggest that the rate of conduction became slower when the disturbance was reduced in size. The rate decreased continuously as the narcosis became deeper, but it decreased without reference to the length of nerve over which the rate is measured. These experiments do not furnish evidence of much value, as the rate of conduction could not be measured accurately enough. However, they do show that the rate of conduction is never obviously dependent on the size of the disturbance, and to this extent they confirm the rest.

So far all the experiments seem to lead to the conclusion that the rate of conduction is independent of the size of the propagated disturbance. It remains to consider the possible sources of error to which the method is liable. These are discussed in the following section.

Possible sources of error.

The first objection which might be raised is one which has been discussed in connection with Exp. 7. It depends on the fact that we do not know how the rate of recovery of the nerve is related to the size of the disturbance. It may be that the nerve recovers more rapidly from a small disturbance than it does from a large one. If this is so, the nerve will recover more rapidly from a disturbance which has travelled a considerable distance in a region of decrement than it will from a disturbance which has travelled a short distance only, and therefore we do wrong to compare the rates of conduction of the two disturbances on the assumption that the nerve recovers from both at the same rate. This objection would be valid enough if the interval for muscular summation had been measured with the second stimulus applied at a point actually within the region of decrement, for in this case the size of the first disturbance at this point would certainly depend on the distance it had travelled, and therefore we should not be justified in assuming that every disturbance set up a refractory period of the same length. However, in actual fact this difficulty does not arise; the second stimulus is not sent in at any point within the narcotised region, but at a point on the normal nerve between the narcotised region and the muscle, namely on the muscle side of the vaseline plug closing the slot *a*. This point was never affected by the narcotic, for its excitability remained normal after conduction had been suspended completely in the rest of the nerve, also the interval for muscular summation with both stimuli at *a* was never increased however deeply the nerve might have been narcotised. It has been shown that a disturbance regains its normal size when it leaves a region of decrement and re-enters normal nerve[1]. Thus all disturbances which come from the narcotised region and reach the electrode *a* must be the same size when they arrive there, whatever their size may have been when they were on the point of leaving the region of decrement. Consequently the nerve at *a* will take the same time to recover from any disturbance, whatever the point at which the disturbance was set up. For this reason the experiment is not invalidated by the fact that we do not know the relation between the size of the disturbance and the length of the refractory period it sets up.

As a matter of fact we should arrive at the same conclusion (*i.e.* that the rate of conduction does not depend on the size of the disturbance) even if we refused to admit that the disturbance regained its normal size when it left the region of decrement. In this case, it is true, the present experiments would be invalid. But Fröhlich has shown that a disturbance regains its normal rate of conduction when it leaves the affected region. If it does not at the same time regain its normal size, it is evident that a disturbance of subnormal size must be conducted at the normal rate when it emerges into normal tissue. Therefore the rate of conduction is not reduced although the size of the disturbance is subnormal, and so the rate cannot depend on the size of the disturbance.

Another objection might be raised on the following grounds. According to Lodholtz[2], the size of the disturbance in narcotised nerve depends upon the strength of the stimulus which set it up. This is deduced from the fact that the strength of stimulus necessary to affect the muscle increases as the seat of stimulation is shifted higher up the nerve, *i.e.* as the reduction suffered by the disturbance on its course down the nerve becomes greater and greater. If this view is correct, it is clear that the threshold stimulus at a peripheral electrode sets up a much smaller disturbance than that set up by the threshold stimulus at a central electrode. This would lead to considerable difficulties in interpreting the results of the experiment if the first stimulus had been of threshold strength, or some multiple of it, at each electrode. However, the first stimulus was always of the same strength and it was not adjusted separately for each electrode. As a rule it was twice the strength required to excite at the least excitable point. Thus we have no reason to suppose that there was any difference in the size of the disturbances set up at the different electrodes. In any case the objection is done away with by two experiments which were made with the express purpose of finding the relation between the strength of the stimulus and the rate of conduction of the disturbance. In the first (Exp. 9) the rate of conduction before narcotisation was 33·4 metres per sec., and after narcotisation it was 9·4 ± ·15 metres per sec. whether the stimulus was 1·4 times or 4·7 times its threshold value. In the other the rate was originally 31·2 metres per sec. and this was reduced to 9·15 ± ·15 metres per sec. whether the stimulus was 1·4 or 7·3 times the threshold value. In both experiments the conduction time was measured from the electrode *f*, and the nerve was conducting with a decrement. These experiments show that one of two alternatives must be true. Either Lodholtz is mistaken in supposing that the size of the disturbance set up in narcotised tissue depends on the strength of the stimulus, or else, if he is right, the size of the disturbance cannot have any influence on the rate of conduction. In either case the objection must be set aside.

---

[1] Adrian, This *Journal*, XLV. p. 389. 1912.

[2] *Ztsch. f. allgm. Physiol.* XV. p. 316. 1913.

It remains to consider the form in which the conclusion may be stated. The rate of conduction seems to remain constant within the limits of experimental error although the size of the propagated disturbance is considerably reduced. Are we therefore justified in concluding that the rate of conduction never depends on the size of the disturbance? Certainly it is conceivable that the rate is not affected as long as the disturbance is greater than a certain size, but that once it is reduced below this size the rate of conduction begins to be affected. There is nothing in the foregoing experiments to disprove this possibility, but it is clear that the limiting size, if it exists, must be extremely small. In many cases the rate was measured from the electrode *f* at a time when all stimuli at *g* failed to affect the muscle. Under these circumstances the disturbance starting from *f* must have been reduced to such an extent that it would have been extinguished completely if its path in the narcotised region had been 20 °/₀ longer. Therefore if the rate of conduction is ever affected by the size of the disturbance, it can be only when the disturbance is so small as to be on the verge of extinction.

SUMMARY.

If the rate of conduction in nerve depends on the size of the disturbance which is conducted, we should expect to find the rate decreasing continuously as the disturbance passes through a region of decrement. According to Koike, the rate of conduction in a narcotised region is uniform and does not become slower and slower as the disturbance is reduced in size. Fröhlich disputes this conclusion. In the present experiments the rate of conduction was measured by Lucas' method, which consists in determining the time intervening between the stimulus and the recovery of the nerve at different distances from the seat of stimulation. The nerve was narcotised with alcohol dissolved in Ringer's fluid. It was found that the rate of conduction did not depend on the distance travelled by the disturbance, although the disturbance suffered a considerable decrement as it passed down the nerve. This confirms Koike's conclusion that the rate of conduction does not depend on the size of the disturbance.

The rate of conduction in the narcotised area does not vary by as much as 3·5 °/₀ when the strength of stimulus is increased to seven times its threshold value. This furnishes an additional proof of the conclusion given above, if we are willing to accept Lodholtz's view that the size of the disturbance set up in narcotised nerve depends on the strength of the stimulus.

THE ANTAGONISM OF CURARI AND NICOTINE IN SKELETAL MUSCLE. BY J. N. LANGLEY, F.R.S.

(*From the Physiological Laboratory, Cambridge.*)

CONTENTS.

		PAGE
1.	The effect of curari given before nicotine on the contraction caused by nicotine in the neural region of the muscle	74
2.	The effect of curari given before nicotine on the contraction caused by nicotine in the general muscle substance	91
3.	The effect of curari given after nicotine	97
4.	The effect of mixtures of curari and nicotine	102
5.	The region of action	102

THE aim of this paper is partly to investigate more closely the laws governing the antagonism of nicotine and curari on striated muscle, and partly to meet the objections which have been taken to my contention that both these poisons so far as regards the tonic contraction produce their effects by an action on the neural region of the muscle and not by an action on the nerve endings.

Method. The usual procedure was to fix the muscle in the chamber in Ringer's fluid. After 5 to 10 minutes the fluid was run off, and curari or nicotine solution added. After a certain time the solution was run off, the muscle and chamber washed with Ringer's fluid, and nicotine or curari added. In a few of the cases in which ·25 to 2 p.c. curari was used before nicotine, the muscle was placed in curari in a glass dish before being fixed in the muscle-chamber. Stock 1 p.c. solutions of curari[1] and nicotine were made up in Ringer's fluid containing NaCl ·65, CaCl ·025, KCl ·02; from these more dilute solutions were made as required.

Description of figures. Except in Figs. 1 and 2 the effects on the two muscles of the same frog are shown. Unless otherwise mentioned the load was 3 grams. The time is marked in 10 second intervals. When the magnification is given, it refers to the magnification of the actual shortening of the muscle. After taking a tracing for a few minutes

---

[1] Curari from the same jar has been used in all my experiments.

(usually 3), the drum was stopped and a short tracing (30 secs.) taken at intervals; the duration of the several intervals is given on the tracings.

In a preliminary communication[1] in 1909, I briefly described some results regarding the mutual antagonism of nicotine and curari upon the sartorius, the flexor carpi radialis, and the rectus abdominis muscles. I mentioned that the percentage of curari required to prevent nicotine from causing an immediate contraction varied directly with the percentage of nicotine up to ·1 p.c., and that the percentage of curari required to prevent ·1 p.c. nicotine from causing contraction was about ·001 p.c. in the sartorius, ·5 p.c. in the flexor carpi radialis, and ·25 in the rectus abdominis, *i.e.* the percentage varied directly as the responsiveness of the muscle to nicotine. Further experiments were made on all three muscles, and the results on the sartorius were published[2] in the same year. The completion of the investigation was delayed by other work. On taking it up again, I found that the rectus abdominis muscle was the more satisfactory muscle to use, and in consequence I have made comparatively few further experiments with the flexor carpi radialis. The older observations were many but they may be treated briefly.

1. THE EFFECT OF CURARI GIVEN BEFORE NICOTINE ON THE CONTRACTION IN THE NEURAL REGION OF THE MUSCLE.

As has been shown earlier, the immediate contraction caused by nicotine up to about ·1 p.c. is confined to the neural region of the muscle. This section, then, deals with the effect of curari when given before ·0001 p.c. to ·1 p.c. In a curarised muscle, nicotine may or may not cause contraction at once, when it does it will be convenient to speak of the contraction occurring during the first few minutes as the immediate contraction, and that which follows on this as the subsequent contraction. When nicotine does not cause an immediate contraction it may still cause one at some later time; it will be convenient to speak of this as the delayed contraction.

(*a*) *The flexor carpi radialis and the rectus abdominis muscles.*

In the older experiments, the muscles were placed in different percentages of curari, and the effect of different percentages of nicotine observed. The two muscles of each frog were not consistently used, so that some caution was required in drawing inferences from differences in the curves. But it was clear that a given low concentration of curarine prevented a certain low concentration of nicotine from causing immediate contraction, and that as the concentration of nicotine was increased the rate of contraction became greater but did not become approximately normal till the concentration was a large multiple of that just causing contraction. Further it was clear that the greater the concentration of curarine the greater the concentration of nicotine required to produce any contraction. The curves obtained from the two muscles were similar in general features, and it will be sufficient to reproduce examples from one series made on the rectus abdominis.

---

[1] *Proc. Physiol. Soc.* p. lxxi. 1909 (This *Journal*, XXXVIII.).

[2] This *Journal*, XXXIX. p. 235. 1909.

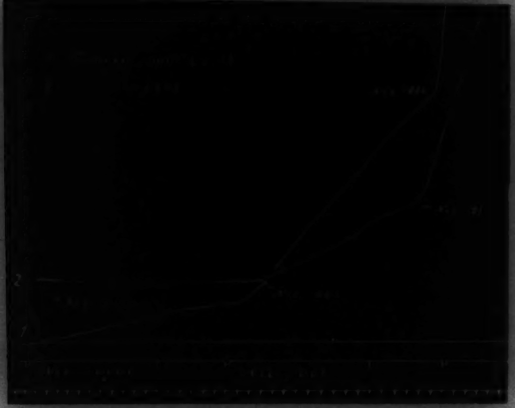


Fig. 1. Rectus abdominis. Reduction of nicotine contraction by previous immersion in ·0001 p.c. and ·0005 p.c. curari for 15 minutes. The time tracing (in 10 secs.) is for the lower tracing. That of the upper tracing was a little slower; the time the muscle was in the nicotine solution was the same in the two cases.

In Fig. 1, it will be seen that curari ·0001 p.c. reduced the rate of contraction caused by ·0001 p.c. nicotine and by subsequent ·001 p.c. nicotine, and that curari ·0005 p.c. prevented ·0001 p.c. nicotine from causing a contraction and reduced the rate of contraction caused by subsequently applied ·001 and ·01 p.c.

The effect of higher percentages of curari is shown in Fig. 2. It will be seen that ·01 p.c. curari reduced the rate of contraction caused by ·01 p.c. nicotine and by subsequent ·1 p.c. nicotine; that curari

·05 p.c. prevented ·01 p.c. nicotine from causing an immediate contraction and that ·25 p.c. curari nearly abolished the immediate effect of ·1 p.c. nicotine.

Fig. 2. Rectus abdominis. Reduction of nicotine contraction by previous immersion in ·01 p.c., ·05 p.c., and ·25 p.c. curari for 15 minutes.

Fig. 3. Flexor carpi radialis. *a* in Ringer's fluid for 30 minutes, *b* in curari ·1 p.c. for 30 minutes. Each stimulated with induction shocks, and nicotine ·01, ·1, and 1 p.c. successively added at intervals of 2 minutes. Then stimulated with induction shocks, secondary coil at 4 and at 0, and with a tetanising current (*t.c.*) $c=0$. D=depress lever to base line.

It was found that immersion of the flexor carpi radialis muscle for 15 minutes in ·1 p.c. curari always prevented ·1 p.c. nicotine from causing contraction[1]. Fig. 3 shows the effect of applying ·01, ·1 and 1 p.c. nicotine successively to a normal muscle and to one which had been in ·1 p.c. curari for 30 minutes. It was also found that ·05 p.c. curari was usually sufficient to produce the same result, though sometimes there was a slight slow contraction; and that ·005 p.c. and ·0005 p.c. curari had a similar relation to ·01 and ·001 p.c. nicotine.

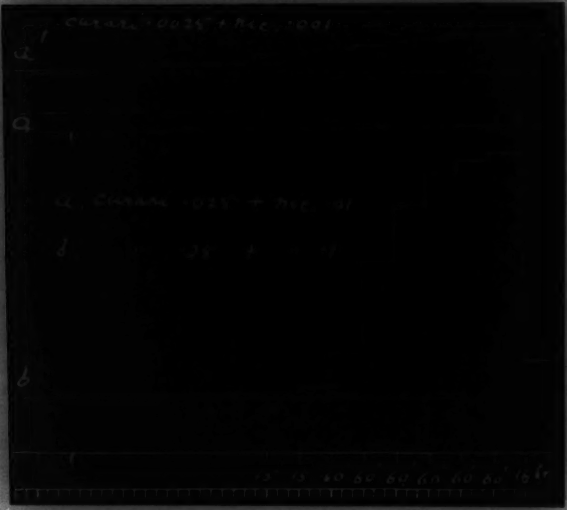


Fig. 4. × 2. Rectus abdominis. Effect of mixtures of nicotine and curari after curari; *a* and *b* from one frog, *a'* from another, *a'* in ·0025 p.c., *a* in ·025 p.c., *b* in ·25 p.c. curari for 15 minutes, then in the mixtures given on the tracing.

Thus in general, the immediate contraction caused by a solution of nicotine of given percentage was prevented by a solution of curari of half that percentage which had acted for 15 minutes.

In the rectus abdominis muscle it was found that if the percentage of curari was 2½ times that of the nicotine, the nicotine had as a rule no immediate effect. In experiments made at different times the ratio varied from 1 to 3, the variation may in part have been due to experiments being made at different times after the preparation of the stock 1 p.c. solution of curari. Since in these experiments the curari solution was removed from the muscle-chamber and the muscle washed with Ringer's fluid, before nicotine was added, the curari taken up by the muscle would, unless firmly combined, gradually diffuse into the nicotine solution. It remained then to ascertain whether curari would prevent indefinitely a nicotine contraction if the concentration of curarine were maintained. The experiments were made by leaving the muscle, loaded with 3 grams, first in curari and then in curari plus nicotine. It was found that the dilute solutions of nicotine if they caused no immediate contraction caused none for several hours (cp. Fig. 4 *a*) and often none during the survival life of the muscle. With ·1 p.c. nicotine there was contraction after a variable time, its extent varied considerably and was sometimes great as in Fig. 4 *b*.

---

[1] The salts of 1 p.c. curari (the residue after the organic substances had been burnt off) made up with Ringer's fluid to the volume of the curari solution taken, had no appreciable effect after 15 minutes' action upon the contraction caused by ·1 p.c. nicotine.

Since nicotine in proportion to its concentration shortens the survival life of the muscle, some late contraction of the muscle due to an action on the general muscle substance is to be expected. When an uncurarised muscle loaded with 3 grams is immersed in nicotine, a rigor rise in the curve is usually seen with ·1 p.c. nicotine, but is not usually seen with ·01 p.c.[1] and the extent of the rise varies considerably in different cases. We may then conclude that the variable rise which occurs on prolonged stay in ·1 p.c. nicotine, and sometimes with a less percentage, is due to an action on the general muscle substance, and that if a given concentration of curarine prevents a given concentration of nicotine from producing an immediate contraction it will, as regards the special action in the neural region, prevent the nicotine from causing contraction at any later time.

When the muscle is kept throughout in curari, but in insufficient percentage to prevent nicotine from causing an immediate contraction, the subsequent contraction, as would be expected, is retarded. The slow, protracted contraction which occurs when the curari is just insufficient to prevent a nicotine effect is shown in Fig. 4 *a'*.

The ratio of the percentage of curari to that of nicotine which just suffices to prevent nicotine from causing a contraction may conveniently be called the *equivalent percentage ratio*[1]. The numerical value of this depends on several factors and amongst them it depends upon the duration of the action of curari (cp. p. 83). But in the corresponding muscles of different frogs, when the duration of the action of curari is the same and not too prolonged, the equivalent percentage ratio is approximately constant, whatever the absolute percentage within the limits dealt with in this section. Thus, as shown above, when the curari is allowed to act for 15 minutes, the equivalent percentage ratio is approximately ½ in the flexor carpi radialis and 2½ in the rectus abdominis.

---

[1] Cp. Langley. This *Journal*, p. 173. 1913. The common absence of rise with ·01 p.c. nicotine in the given conditions does not show that no rigor rise is occurring, but only that if it is occurring it is too slow to counterbalance the coincident relaxation in other molecular aggregates.

Whilst in the earlier experiments there was evidence that the rate of contraction produced by nicotine depended upon the relative percentage of the two substances, the conditions were not such as to bring out the degree of accuracy with which this occurs. This question I have investigated more recently on the rectus abdominis muscle.

Solutions of curari and nicotine were taken in approximately their equivalent percentage ratios, and each solution was diluted with Ringer's fluid 10, 100, and 1000 times. A muscle was placed in each curari solution for 15 minutes, washed with Ringer's fluid, and then immersed in the corresponding solution of nicotine. The following protocol will serve to show the details of the method.

EXP. 1. The following solutions were prepared:

Curare	·25 p.c.	·025 p.c.	·0025 p.c.	·00025 p.c.
Nicotine	·1	·01	·001	·0001

Two rectus abdominis muscles *a* and *b* were fixed in the muscle-chamber and left in Ringer's fluid for 5 mins., then the fluid was run off and curari ·25 p.c. added to *a* and curari ·025 p.c. to *b*. After 15 mins. the curari was run off and the muscle washed three times with Ringer's fluid; the washing took 3 mins. Nicotine ·1 p.c. was then added to *a*, and nicotine ·01 p.c. to *b*.

Another pair of muscles *a'* and *b'* were similarly treated except that *a'* was placed in ·0025 p.c. curari and subsequently in ·001 p.c. nicotine, and *b'* was placed in ·00025 p.c. curari and subsequently in ·0001 p.c. nicotine. Fig. 5 shows the rate and degree of shortening in each case.

It will be seen from Fig. 5 that the effect of ·1 p.c. and of ·01 p.c. nicotine is practically identical, and that the effect of ·001 p.c. and ·0001 p.c. nicotine on another pair of muscles differs but little from that of ·1 and ·01 p.c. The differences which are shown may safely be referred partly to intrinsic differences in the muscles, and partly to the experimental difficulties, for example that of cutting out muscles with exactly the same amount of tissue laterally. In fact the differences observed with different percentages of nicotine are not constant; sometimes the stronger solution, sometimes the weaker one has a slightly greater effect. As an example another experiment may be given.

---

[1] When pure curarine is obtainable the equivalent percentage ratios of curari and nicotine can readily be converted into the equivalent concentration ratios of curarine and nicotine.

Exp. 2. The muscles were treated as follows:

Frog 1. *a* curari ·2 p.c., then Ringer's fluid followed by nicotine ·1 p.c.
b „ ·02 „ „ „ „ „ ·01
Frog 2. *a* „ ·002 „ „ „ „ „ ·001
b „ ·0002 „ „ „ „ „ ·0001

The results are given in Fig. 6.

Fig. 5. × 2. Rectus abdominis. Effect of nicotine after curari, with varying absolute percentage but in the same percentage ratio. See Text.

In this case it will be seen that the primary effect of ·001 and of ·0001 p.c. nicotine was practically identical, but that a slight difference in rate of contraction was caused by ·1 and ·01 p.c.

We may conclude then that in any one muscle the effect of nicotine after curarine is accurately dependent upon their relative concentration, in such way that the rate of the immediate contraction varies inversely as the concentration of curarine and directly as the concentration of nicotine, and that up to a certain limit the rate of contraction is independent of the absolute concentration of the two substances. In other words the reaction velocity in a curarised muscle when transferred to nicotine is in its early stage the same with ·0001 to ·1 p.c. nicotine, if the ratio of the concentration of the curarine to that of the nicotine is constant.

In the preceding paragraph I have spoken of the immediate contraction only. The subsequent contraction is less simple since as mentioned above (p. 78) the muscle gradually passes into rigor, and the time of onset and rate of rigor are very variable. Thus when the absolute percentages of curari and nicotine are varied but the ratio of their percentages kept constant, the occurrence of rigor will tend to make the curves of contraction different. This is of course more likely to occur, the higher the percentage of nicotine. The most marked instance I have seen of what I take to be an early action of ·1 p.c. nicotine on the general muscle substance is given in Fig. 7. But in many cases with ·0001–·01 p.c. nicotine, and in some cases with ·1 p.c., the curves of contraction are very similar for several hours (see Fig. 6 cp. also Fig. 5) and may be similar throughout, so that in all probabilitiy the reaction velocity in the given circumstances is the same not only for the immediate contraction, but also for the subsequent contraction except so far as this is upset by the occurrence of rigor.

Fig. 6. ×1½. As Fig. 5. See Text.

What has just been said of the subsequent contraction holds also for the delayed contraction which begins sooner or later when a muscle is transferred from curari to nicotine.

Fig. 7. ×1¼. As Fig. 5 but showing early rigor rise with ·1 p.c. nicotine.

Fig. 8. ×1⅜. Rectus abdominis. Varying duration of action of ·0005 p.c. curari. See Text. The beginning of the rise of the curve shows the moment of immersion in nicotine.

Fig. 9. ×1⅜. Rectus abdominis. Varying duration of action of ·005 p.c. curari. See Text. It will be noticed that curari had a much greater effect on one pair of muscles than on the other pair.

The time factor in the action of curari. I have previously shown in the sartorius muscle[1] that very dilute solutions of curari have a progressive paralysing action on the contraction caused by nicotine in the neural region of the muslces. The rectus abdominis muscle is much better adapted than the sartorius for observations on the rate of action of different percentages of curari. In Fig. 8 an example is given of the effect of a very low concentration of curarine. The six muscles of three frogs of approximately the same weight and condition were prepared, and left for different times in ·0005 p.c. curari. The times were 1 *a*, 5 minutes, 1 *b*, 1 hour; 2 *a*, 2 hours, 2 *b*, 4 hours; 3 *a*, 16½ hours, 3 *b*, 17½ hours and then in Ringer's fluid for 5 hours. At the end of the given time each was immersed in ·01 p.c. nicotine. It will be seen that the rate of contraction caused by ·01 p.c. nicotine decreased from 1 *a* to 3 *a*, *i.e.* the decrease was greater the longer the action of curari. That the decrease was not due to coincident longer stay in Ringer's fluid is shown by 3 *b*, this was left in curari for the longest period viz. 17½ hours, and Ringer's fluid was then substituted for curari and left for 5 hours. As will be seen a considerable part of the paralysing effect of curari disappeared during the stay in Ringer's fluid.

The tracings obtained do not allow an accurate curve to be drawn showing the extent of the action of curari at equal intervals of time, but it is obvious that the effect proceeds much more rapidly at first than it does later.

With somewhat stronger solutions of curari, the maximal effect producible by the solutions is approximately attained in a shorter time. Fig. 9 shows the result of an experiment in which the duration of action of ·005 p.c. curari was varied.

Exp. 8. Two pairs of rectus abdominis muscles *a*, *b* and *a′*, *b′* were left in ·005 p.c. curari for the following times:

a	1 hour	*a′*	2 hours
b	16 hours	*b′*	4 ,,

Each was washed with Ringer's fluid (2 mins.), and then immersed in ·1 p.c. nicotine.

It will be seen that after ·005 p.c. curari had acted for 1 hour, longer duration of action had a slight, but only a slight, effect[2].

It might then be expected that with still stronger percentages of curari, there would be an easily ascertainable time limit, after which longer stay in curari would produce no further effect. I have made some experiments with ·1 p.c. The results have not been constant, but in most cases the effect of nicotine was less when the muscle was immersed for 2 or 3 hours in curari, than when it was immersed for 15 to 30 minutes. The curves of one such experiment are given in Fig. 10. But there is some uncertainty in the interpretation of the results for ·1 p.c. curari shortens survival life and causes a slow decrease of irritability to nicotine; moreover the curari absorbed and adsorbed by the muscle as a whole, increases up to an unknown limit with increase in duration of immersion, and the action of nicotine may be thereby delayed. The fact however remains that satisfactory evidence of the establishment of equilibrium in the reaction between curari and the tissue was not obtained.

---

[1] This *Journal*, XXXIX. p. 258. 1909.

[2] The tracing also illustrates the difference in reaction which may occur in the corresponding muscles of different frogs.

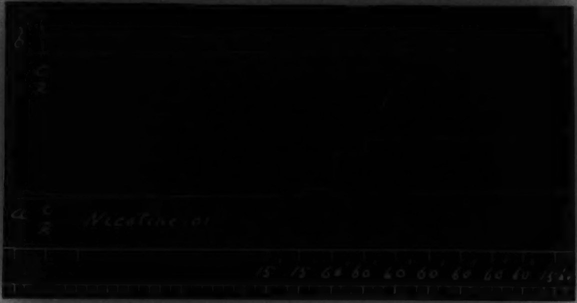


Fig. 10. ×2. Rectus abdominis. Varying duration of action of ·1 p.c. curari. *a*. curari ·1 p.c. 15 minutes; washed twice with Ringer's fluid (5 minutes), then immersed in ·01 p.c. nicotine. *b*. curari ·1 p.c. 3 hours, then treated as *a*.

Reversibility of curari action. The gradual disappearance in the body of the paralysing action of curari indicates, though it does not definitely show, that the action is reversible. Reversibility in survival muscle is shown in Fig. 8 (cp. 3 *a* and 3 *b*). In this case the curari used was only ·0005 p.c. But with all percentages of curari, the paralysing action decreases fairly rapidly on removing the muscle to Ringer's fluid. Thus if one muscle is left in ·1 p.c. curari for 15 minutes, ·01 p.c. nicotine causes no immediate contraction, but if the muscle after being placed in curari is left in Ringer's fluid for 3 hours, a slow contraction is obtained (cp. Fig. 11). The contraction is naturally greater and more rapid if ·1 p.c. nicotine is used instead of ·01 p.c. (cp. Fig. 12 *b*). The rate of recovery after immersing the muscle in nicotine and curari solutions of approximately equivalent percentage ratio cannot very satisfactorily

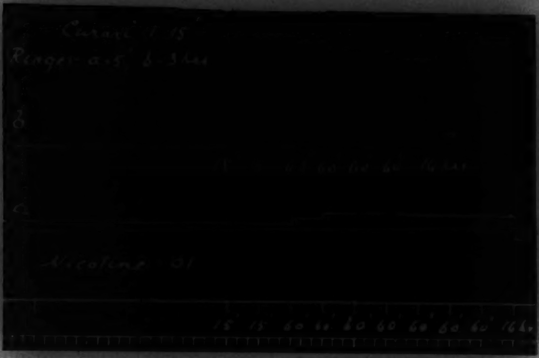


Fig. 11. × 2. Rectus abdominis. Lessening of curari action on stay in Ringer's fluid. See Text.

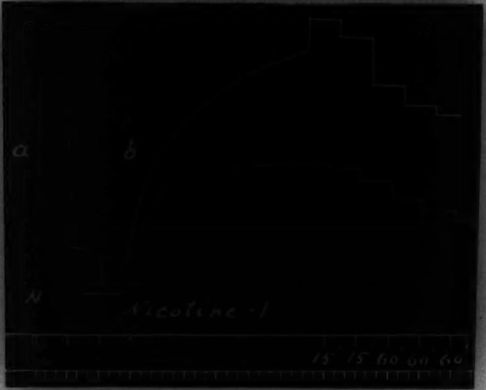


Fig. 12. × 2. Rectus abdominis. Reversal after curari and after nicotine. *a*. Nicotine ·04 p.c. 15 minutes. *b*. Curari ·1 p.c. 15 minutes. Both then washed twice with Ringer's fluid, and left in the fluid for 3 hours. (Curve *b* begins after the stay in the fluid, it started from the same level as *a*.) An additional load of 7 grams was added to *a* for 5 minutes in order to stretch it, then each muscle was immersed in ·1 p.c. nicotine.

be compared on account of the contracture produced by the nicotine, but the actual shortening caused by nicotine is greater in a muscle which has been treated with curari and then with Ringer's fluid, than in one which has been treated with nicotine and then with Ringer's fluid (cp. Fig. 12).

(*b*) *The sartorius muscle.*

In the observations on the sartorius muscle which I have described earlier[1], I found that a stay of 15 minutes in ·0001 p.c. curari very nearly prevented ·01 p.c. nicotine from causing contraction and that a like stay in ·001 p.c. curari usually prevented ·1 p.c. nicotine from causing contraction, *i.e.* the equivalent percentage ratio in these conditions was $\frac{1}{50}$–$\frac{1}{100}$. In the observations I have made in the last two years, the ratio generally varied within these limits, but was more frequently about $\frac{1}{50}$ than $\frac{1}{100}$. We have seen earlier that the corresponding ratios in the flexor carpi radialis and rectus abdominis muscles are roughly $\frac{1}{4}$ and $2\frac{1}{2}$ respectively. The differences as I pointed out earlier are obviously related to the degree of responsiveness to nicotine, *i.e.* the more responsive a muscle is to nicotine, the higher the concentration of curarine required to prevent the nicotine contraction.

It follows from this, and from the fact that there is no great difference in the amount of curari required to paralyse the motor nerves of different muscles in the frog, that when the most resistant nerves have been paralysed by curari, a certain percentage of nicotine will cause no contraction in the sartorius, a fairly strong contraction in the flexor carpi radialis, and a still stronger contraction in the rectus abdominis.

The sartorius is very ill adapted to show that when nicotine causes a contraction after curari, the rate of contraction is the same with different concentrations of nicotine provided the ratio of the concentration of curarine to that of nicotine is the same. This is due to several causes. It would hardly be worth while to consider them but for the fact that some have an important bearing on the laws governing the antagonism of curari and nicotine as regards muscular twitchings.

(*a*) The difference between the highest and lowest concentration of nicotine which can be used is very much less in the sartorius than in the rectus abdominis. In the sartorius we are practically limited to comparing the effect of solutions of nicotine ranging from ·005 to ·1 p.c. and sometimes only from ·005 to ·05 p.c. When the percentage of curari is sufficient to diminish the nicotine twitchings so that they do not obscure the tonic contraction, the difference in the curves of tonic contraction caused by ·1 and ·01 p.c. or by ·05 and ·005 p.c. nicotine is slight. Fig. 13 A shows the maximum difference I have obtained with ·1 and ·01 p.c. nicotine after the muscles had been for 15 minutes in ·0005 p.c. curari. In this case the twitchings had been reduced by adding ·1 p.c. $MgCl_2$ to the Ringer's fluid, and the load was 3 grams. In a number of experiments in which the load was only 1 gram, the

---

[1] This *Journal*, XXXIX. p. 252. 1909.

Fig. 13. ×3. Sartorius. Comparative action of nicotine of different percentages after 15 minutes' immersion in curari ·0005 p.c. In *A*, load 3 grams; in *B*, load 1 gram.

Fig. 14. ×4. Sartorius, Different forms of rigor rise with ·1 p.c. nicotine. *a* and *b* from one frog, *c* from another. Load 2 grams.

difference was less (cp. Fig. 13 B) and not infrequently it was barely perceptible.

(*b*) I have said that the percentages of nicotine which can be used range sometimes from ·005 to ·05 p.c. only. This occurs when ·1 p.c. nicotine causes a slight immediate contraction in the general muscle substance (cp. Fig. 14). An action on the general muscle substance can be told by the contraction continuing for two or more hours.

The slow shortening caused by ·1 p.c. nicotine may gradually diminish for a time and then be followed by a second shortening, or it may continue till the muscle dies (Fig. 14). The variations in the curve no doubt depend on the extent to which the muscle contains fibres which are more readily affected than others. This slow rise is not delayed by increasing the duration of action of curari, nor by increasing its percentage. Thus the rate of rise is as great in a muscle which has been treated with ·1 p.c. curari as in one treated with ·002 p.c. (cp. Fig. 15), indeed in most of my experiments the rate of rise was slightly greater when the stronger solution of curari had been used.

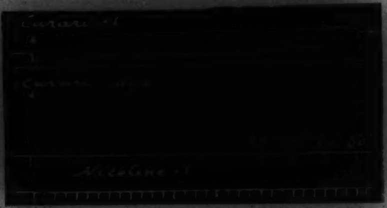


Fig. 15. × 4. Sartorius. Absence of effect of curari on the small initial contraction sometimes caused by ·1 p.c. nicotine in the general muscle substance. Load 4 grams.

(*c*) The more frequent cause of difficulty in the observations is the occurrence of twitchings[1] which more or less obscure the tonic contraction. In slightly curarised, as in normal muscles, the twitchings are more prolonged in ·005–·05 p.c. nicotine than in ·05–·1 p.c. and if the curari is insufficient to prevent these, the exact degree of tonic contraction with the weaker and stronger nicotine solutions cannot be determined. Fig. 16 illustrates several of the difficulties which occur. The ratio of the percentage of curari to that of nicotine was the same in both muscles. The strong initial contraction may have been of the kind which is sometimes produced when the fluid run in is physiological saline solution. The protracted twitchings with ·01 p.c. nicotine make it impossible to say whether there was any tonic contraction. The persistent contraction after ·1 p.c. nicotine is almost certainly caused by an action on the general muscle substance. Probably in neither muscle was there any tonic contraction.

---

[1] It may be mentioned that in general the twitchings are increased by a prolonged stay in the Ringer's fluid used.

The percentage of curari required to prevent nicotine from producing twitchings varies in different muscle. From January to March 1913 the twitchings obscured the tonic contraction in nearly every experiment (about 20). In a previous paper I have mentioned that in sartorius muscles it is usual, especially if they are not removed from the body soon after death, for curari to paralyse the nicotine twitchings a little earlier than it paralyses the nicotine tonic contraction. In the

Fig. 16. ×3. Sartorius. Effect of nicotine after curari; the ratio of the percentages of the two drugs being the same, but the absolute percentage different. Each muscle in curari for 15 minutes.

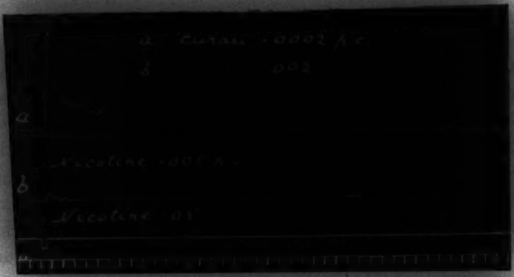


Fig. 17. ×4. Sartorius. Muscular twitchings caused by nicotine after curari; the two drugs being in approximately equivalent percentage ratio as regards the tonic contraction, but the absolute percentage being different. Each muscle in curari for 15 minutes.

experiments just mentioned the reverse was commonly the case. Thus in Fig. 17 there was faint twitching with ·05 p.c. nicotine after ·002 p.c. curari, and without a trace of tonic contraction; probably indeed ·001 p.c. curari would have been sufficient to prevent the latter.

When the factors mentioned above are taken into account, the results I have obtained give reasonable evidence that the rate of the nicotine contraction in the sartorius, as in the rectus abdominis, depends on the relative concentration of the curarine and nicotine. The more conclusive curves are those in which there is no twitching. In one series of experiments I added $MgCl_2$ to all the fluids used. This reduces the twitchings[1] more than it reduces the tonic contraction. Fig. 18 gives a pair of the curves obtained. It will be seen that the contraction with ·01 p.c. nicotine after ·0001 p.c. curari is very nearly the same as that given by ·1 p.c. nicotine after ·001 p.c. curari.

Fig. 18. ×4. Sartorius. Effect on tonic contraction of nicotine after curari, the two being in approximately equivalent percentage ratio but in different absolute percentage. All the solutions contained $MgCl_2$ ·05 p.c.; *a* in curari ·0001 p.c., *b* in curari ·001 p.c. each for 15 mins. before being placed in nicotine. Load 2 grams.

The production of tonic contraction by nicotine and the production of twitchings present some obvious points of difference. The rate at which the tonic contraction proceeds and its strength are proportional to the concentration of the nicotine. We cannot properly speak of the rate at which the twitchings proceed, nor can we speak of the strength of the twitch in the sense in which we speak of the strength of the tonic contraction. It is possible that the twitch set up in any one fibre is on the "all or none" principle and that its strength does not vary with the concentration of the nicotine. The strength of the twitch of the muscle may depend wholly, as it clearly does in part, on the number of fibres in action. We have then for comparison, only the number of twitches set up, and the means of judging of these is very imperfect. It is clear that when the concentration of nicotine is increased beyond a certain point, the time during which twitches occur in the muscle decreases, and it is probable that the number decreases on either side of an optimal concentration.

---

[1] In these experiments when the solutions contained $MgCl_2$ up to ·05 p.c. the twitchings were rather greater with the more dilute nicotine, when the $MgCl_2$ was ·1 to ·5 p.c. the twitchings were rather greater with the less dilute nicotine.

From these considerations we may I think conclude that the antagonism of curari for nicotine as regards the twitchings cannot follow the law which holds for the tonic contraction. In fact the figures given above (see Figs. 16, 17) show that the extent of the reduction of the nicotine twitchings by previous application of curari does not depend —at any rate within wide limits—upon the ratio of the concentration of curarine to nicotine, but upon the absolute concentration of the curarine. If the latter is insufficient to prevent ·1 p.c. nicotine from causing twitchings, it is also insufficient to prevent ·005 p.c. from causing twitchings.

The curves suggest that the effect of the stronger nicotine is prevented before that of the dilute. I have not found any case in which this occurred, but observations on this point are better made by direct examination with a dissecting lens, and of these I have made a few only.

It seems probable that the antagonism by curari of the twitchings caused by physostigmine in mammalian muscle follows the rule given above for the antagonism by curari of the nicotine twitchings in frog's muscle.

2. THE EFFECT OF CURARI GIVEN BEFORE NICOTINE ON THE CONTRACTION IN THE GENERAL MUSCLE SUBSTANCE.

As the nicotine percentage is increased above ·1 p.c. a point is soon reached at which contraction is caused throughout the whole muscle fibre[1]. We have then to investigate whether in this case the nicotine action is antagonised by curari and if so whether the antagonism follows the same laws as those which hold when the neural region is alone affected.

From what has been shown above we may safely infer that if the ratio of the percentage of curari to that of nicotine is equal to, or greater than, the equivalent percentage ratio as determined for the lower percentages, an immediate contraction will not be caused by a special action on the neural region, and that if contraction is obtained it is caused by an action on the general muscle substance. We may infer further, that the ratio of the percentage of curari to that of nicotine may be considerably below the equivalent percentage ratio, yet the immediate contraction caused by nicotine will still be but slight. In considering the effect of the higher percentages of curari, it is to be borne in mind that since the curari is dissolved in Ringer's fluid the solutions are more or less hypertonic, and that other substances than curarine which are present in curari may alter the condition of the muscle. It is also to be remembered that the rigor rise with nicotine is much more variable than the nicotine contraction of the neural region, and that the two muscles of a frog taken straightway after death, may show marked differences.

---

[1] The minimal percentage of nicotine which causes an immediate contraction by an action on the general muscle substance varies in individual cases, and most of my observations on this question have been incidental. But there can I think be no doubt that the threshold of nicotine stimulation of the general muscle substance is higher in the rectus abdominis than in the sartorius.

With regard to the former point, one or two additional facts may be mentioned. In the rectus abdominis muscle, even ·1 p.c. curari causes in time some shortening (cp. Fig. 10 *b*); this shortening increases with the percentage of the curari. An example of the extent of shortening with 1 p.c. curari is given in Fig. 19, in *a* the muscle was left in 1 p.c. curari for 15 mins., and in *b* for 60 mins. In consequence of this effect, the longer the stay in curari, the more the curve will be above the base line, and the less will be the rise in the curve caused by nicotine. A small part only of this shortening of the muscle appears to be due to the inorganic salts of curari, for if a 1 p.c. curari solution is evaporated to dryness, the organic substance charred or burnt off, and distilled water added to restore the original volume, the salt solution so obtained causes much less shortening of the muscle than is caused by 1 p.c. curari. The inorganic salts of curari do however appreciably though slowly reduce the irritability to nicotine, thus after a stay of 5 hours in the salt solution just mentioned the rate of rise of the curve with ·001 p.c. nicotine is approximately that caused by ·0005–·00025 p.c. in the muscle, kept for the same time in Ringer's fluid.

Fig. 19. × 1¼. Rectus abdominis. See Text.

The question is simplest in the sartorius muscle. When curari is only allowed to act for fifteen minutes, the equivalent percentage ratio is low so that it is certain that a solution containing much less than ·1 p.c. curari will prevent ·1 p.c. nicotine from producing an immediate contraction by an action on the special mechanism of the neural region.

I have given in a former paper[1] an account of a number of experiments made on the sartorius, and have shown that an increase in the percentage of curari from about ·01 to 1 p.c. increases rather than decreases the immediate effect of ·5 and of 1 p.c. nicotine. It follows that within these limits curari does not antagonise to an appreciable extent the action of nicotine on the general muscle substance.

At the time of the experiments on the sartorius muscle I also made experiments on the flexor carpi radialis and rectus abdominis muscles, but they were nearly all made on the muscles of different frogs, so that the curves could only be compared in their broad features. Since the reaction of the two muscles is much alike, I have only repeated the crucial experiments on the rectus abdominis muscle. With regard to the flexor carpi radialis it is sufficient to say that 1 p.c. nicotine still causes an immediate contraction after the muscle has been immersed for 30 minutes in 1 p.c. curari, so that the action of 1 p.c. nicotine on the general muscle substance is not prevented by a percentage of curari which completely prevents it from causing contraction by way of the special mechanism of the neural region.

In the observations on the rectus abdominis one form of experiment was as follows:—a series of solutions of curari and nicotine were made of different percentages, the ratio of the percentage of curari to that of nicotine being the same in all and approximately that of the equivalent percentage ratio. The effect of the solutions was determined on pairs of muscles. For example

EXP. 4. Curari solutions 2, 1·5, 1, ·5, ·25 and ·025 p.c.
Nicotine ,, ·8, ·6, ·4, ·2, ·1 and ·01 p.c.

The right and left rectus abdominis muscles of a frog were placed in 2 p.c., and in 1·5 p.c. curari respectively for 15 minutes, rinsed for about 10 seconds in Ringer's fluid, fastened in similar muscle-chambers, then nicotine ·8 p.c. added to one, and nicotine ·6 p.c. to the other. The muscles of two other frogs were treated similarly with the other solutions. There was no immediate contraction with ·01 p.c., ·1 p.c. and ·2 p.c. nicotine; immediate contraction was caused by the three stronger solutions. The curves obtained with ·6 p.c. and ·8 p.c. nicotine are given in Fig. 20 A, and those obtained in another similar experiment with ·2 p.c. and ·8 p.c. nicotine in Fig. 20 B.

These and other experiments showed that the ratio of curari to nicotine percentage which suffices to prevent an immediate nicotine contraction when the percentage ranges from ·0001 to ·1 p.c. does not prevent an immediate contraction when the percentage of nicotine ranges from about ·4 p.c. to 1 p.c., *i.e.* when the percentage of nicotine is about that which demonstrably causes contraction in the general muscle substance. It will be noticed in Fig. 20 A that the contraction caused by ·8 p.c. nicotine is greater than that caused by ·6 p.c., *i.e.* although the ratio of nicotine to curari percentage is the same in the two cases, the more concentrated nicotine has the greater effect. This always occurs when nicotine affects the general muscle substance, and the fact shows that if curari has any antagonism for nicotine as regards the general muscle substance, the antagonism is of a totally different kind from that which occurs as regards the special mechanism of the neural region. This can be shown in another way, namely, by treating two muscles with different percentages of curari and with the same percentage of nicotine. In such case when the action takes place in the neural region the muscle which has been treated with the higher percentage of curari gives the less contraction, but when the action takes place in the general muscle substance, the contractions are practically equal, such differences as occur being within the limits of experimental error. In the experiments of this kind I have usually used curari ·25, ·5, ·75

---

[1] *Journ. of Physiol.* XXXIX. p. 263. 1909.

Fig. 20. ×2. Rectus abdominis. Action of nicotine on general muscle substance.

and 1 p.c., and nicotine ·4 p.c. The tracing from an experiment is given in Fig. 21.

The simplest explanation of the results is that curari does not antagonise at all the action of nicotine on the general muscle substance. In fact no percentage of curari, unless it kills the muscle, will prevent ·5 to 1 p.c. nicotine from causing an immediate contraction. We may at any rate conclude that if curarine delays the action of nicotine on the general muscle substance the delay is but slight, and that the action is fundamentally different from that which occurs in the neural region.

Fig. 21. ×1½. Rectus abdominis. Time of stay in curari=15 minutes.

There is one other point to consider with regard to the effect of curari on the contraction caused by nicotine. In all muscles the contraction of the general muscle substance passes into rigor, the rate depending on the concentration of the nicotine. In the curarised rectus abdominis, ·25 to 1 p.c. nicotine causes a quick primary rise followed by a slow secondary rise. In the curarised sartorius there is usually after the primary rise either a slight fall or a plateau, before the secondary rise. The form of the curve depends on the percentage of nicotine, and to some extent on the condition of the muscle. In my previous experiments I found[1] that curari strong enough to reduce markedly the irritability of the muscle, reduced the second rise, and I suggested that this effect might be due to the action of the salts of curari and not to curarine antagonising the nicotine action. Burridge[2] has described a

[1] This *Journal*, xxxix. p. 265. 1909. On p. 265 there are two misprints; on lines 5 and 11 from the bottom 'nicotine' should be 'curari.'

[2] *Ibid.* xlii. p. 374. 1911.

definite antagonism as regards the secondary rise. According to him the onset of the secondary rise is delayed in curarised muscles, the delay is very considerable when the immersion in curari is prolonged, and is much increased when curari is added to the nicotine. The muscles were left in about ·135 to ·16 p.c. curari for 3 or 4 hours and then treated with ·5 to 1 p.c. nicotine + ·4 p.c. curari. In the instance given of the hindrance to the action of 1 p.c. nicotine, the delay was 11 mins. This result I did not obtain earlier in somewhat similar experiments and I have since made ten experiments on this point. The two sartorius muscles of a frog were prepared and one placed in ·16 p.c. curari for 1½ to 5 hours, the other in Ringer's fluid for the same time. The mixtures contained (1) ·5 p.c. nicotine and ·2 p.c. curari, (2) ·5 p.c. nicotine and ·4 p.c. curari, (3) 1 p.c. nicotine and ·4 p.c. curari. In none of these cases had curari any effect comparable to that described by Burridge, and I think it may be regarded as certain that if curari interferes with the action of nicotine as regards the secondary rise, its effect is slight, and that there is no specific antagonism such as occurs in the neural region of the muscle.

Fig. 22. × 3. Rigor rise caused by nicotine after prolonged immersion of the sartorius in curari. Load 2 grams.

The curves which were taken at different times of the year varied in form, and this was especially the case with the mixtures containing ·5 p.c. nicotine. One form of curve is given in Fig. 22.

With 1 p.c. nicotine the curves were more constant in form, and there was usually some delay in the curarised muscle in the time of beginning of the secondary rise and retardation of the first part of the rise (Fig. 23). But the effect it will be seen is small and there is no certainty as to its cause. In the uncurarised muscle there is a strong transient contraction in the neural region of the muscle, and the change produced in the general muscle substance in this region may make it more easily affected by strong nicotine. Moreover the addition to the Ringer's fluid of the constituents of curari other than curarine will almost certainly alter slightly the response of the general muscle substance. It may also be noticed that the only evidence that the primary and secondary rises are due to different processes is the fall occurring between the two when the percentage of nicotine exceeds a certain point, and the fall might be due to some of the molecular aggregates being more completely acted on than others, and so more rapidly passing into the stage of 'relaxation,' the processes might then be the same throughout, and the variation in the curves be due to differences in the rate of action on the several molecular aggregates.'

Fig. 23. × 3. Rigor rise caused by nicotine after prolonged immersion of the sartorius in curari. Load 2 grams.

In a few experiments I compared the effects on curarised muscles of ·5 p.c. nicotine, and of a mixture containing ·5 p.c. nicotine and ·4 p.c. curari, and found no certain difference, *i.e.* the addition of curari to the nicotine had no definite effect.

3. THE EFFECT OF CURARI GIVEN AFTER NICOTINE.

In a former paper[1] I have given a brief account of the effect of curari when given after nicotine, and stated that in all cases it puts an end to the stimulating action of nicotine and has little or no effect upon the state caused by the stimulation (the contracture). The experiments were chiefly made upon the flexor carpi radialis and rectus abdominis muscles. Those on the rectus abdominis I have repeated in the past year and a half so that the tracings could be more directly compared with those given of the effect of nicotine on this muscle[2].

---

[1] This *Journal*, xxxix. pp. 267, 268. 1909.

[2] *Ibid.* xlvii. p. 159. 1913.

These I shall describe fully so that it will be sufficient to give a summary of the observations on the flexor carpi radialis.

Flexor carpi radialis. The behaviour of a series of muscles was compared in two ways, in one series the same percentage of nicotine was used and the percentage of curari varied; in the other the percentage of nicotine was varied and a given percentage of curari used. It was found that the rate of relaxation depended upon the relative percentage of the nicotine and curari, upon the duration of the nicotine action and of course upon the load. With a light load, there was a residual contracture little or not at all affected by curari. Unless the percentage of curari was largely in excess of that of nicotine, an abrupt alteration in the curve was not caused by curari.

The rectus abdominis. With solutions of nicotine below ·001 p.c., the maximum height of the contraction is only slowly attained (cp. This *Journal*, XLVII. p. 172). If curari of percentage greater than that of the equivalent percentage ratio is substituted for nicotine during the rise of the curve, the rise is rapidly stopped and gives way to a fall. In Fig. 24, the effect is shown of substituting ·05 p.c. curari for ·00025 p.c. nicotine after the latter had acted for two minutes; the other muscle which was left in the nicotine for an hour gave a practically identical fall. The fall is not always quite as abrupt as in this case, as is shown in Fig. 25, which also shows the slight effect of an hour's immersion in Ringer's fluid before the muscle is placed in curari.

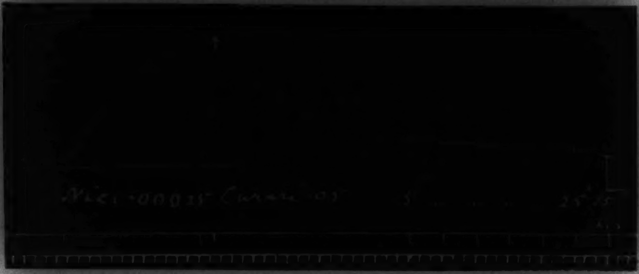


Fig. 24. ×2. Rectus abdominis. Nicotine ·00025 p.c. for 2 minutes, then nicotine run off, and curari ·05 p.c. poured into the muscle-chamber.

The relaxation caused by curari in the early stage of nicotine action is very much prompter and greater than that caused by Ringer's fluid (cp. This *Journal*, XLVII. p. 183, Figs. 16 and 17), *i.e.* the curari solution does not act solely by washing away the nicotine, but has a special antagonising action. Since, as we have seen, curari when given first prevents the nicotine contraction, there can be no doubt that it will stop it whenever it is occurring. The production of a contraction by nicotine I have spoken of as its stimulating action. As contraction is obviously occurring during the rise of the curve, the relaxation caused by curari must be due in part to its putting an end to the stimulating action of nicotine. For a considerable time after the maximum rise has been obtained with dilute solutions of nicotine, curari will cause a markedly faster fall than Ringer's fluid. We may conclude then that with dilute solutions of nicotine, the stimulating action is very prolonged.

Fig. 25. ×2. Rectus abdominis. Effect of ·05 p.c. curari after the muscle had been immersed for 8 minutes in ·00025 p.c. curari.

With higher concentrations of nicotine up to ·01 p.c., which is the highest I have used, a fall is still obtained provided the ratio Cc/Cn is above the equivalent percentage ratio. The higher the concentration of nicotine the less the fall produced by a given concentration of curarine. Thus if one muscle of a pair be immersed in ·001 p.c. nicotine for 5 minutes, and the other in ·01 p.c. for the same time; the relaxation on substituting ·1 p.c. curari is markedly greater in the former than in the latter. With a concentration of nicotine which causes an approximately maximal contraction, the fall caused by curari is less the longer the nicotine has acted. In Fig. 26 one muscle (*a*) was left in ·01 p.c. nicotine for 2 minutes, washed in Ringer's fluid (about 5 mins.), and then immersed in ·1 p.c. curari, the other (*b*) was left in the nicotine for 35 minutes and similarly treated. It will be seen that the fall caused in the first ten minutes was considerably less in the muscle which had been the longer time in nicotine. In this experiment the load was 5 grams. When the action of nicotine ·01 p.c. is of still longer

Fig. 26. ×2. Rectus abdominis. Load 5 grams. See Text.

Fig. 27. ×1½. Rectus abdominis. Nicotine ·01 p.c. for 65 minutes, then washed in Ringer's fluid. *a* immersed in fresh Ringer's fluid at *R*. *b* immersed in ·1 p.c. curari at *C*.

duration, curari ·1 p.c. may make no abrupt change on the curve, but there is a gradual and prolonged fall greater than that which occurs when in Ringer's fluid alone. This is shown in Fig. 27.

But though the relaxation is greater than in Ringer's fluid, the muscle does not usually, with a load of 3 grams, attain its original length in a day. The relaxation is of course less as the load is reduced. It is clear then that curari has very little, if any, effect on the 'contracture.' Whilst direct proof that the curari has no effect on the contracture is wanting, the trend of the evidence is to show that it has none, and that the relaxation occurring is due to the abolition of a slight stimulating action of nicotine. In that case it follows from the experiments that ·01 p.c. nicotine has a trifling stimulating effect after an hour's action and probably for much longer, *i.e.* for a considerable time after the normal relaxation has set in.

Fig. 28. ×1⅓. Rectus abdominis. Load 2½ grams. At *R* muscles for 5 minutes in Ringer's fluid, then immersed in curari.

But when the ratio of the percentage of curari to nicotine is distinctly below the equivalent percentage ratio, curari does not cause an abrupt change in the direction of the curve, even when the muscle is left in the nicotine for 5 minutes only; it causes merely a trifling deflection similar to that caused by stronger percentages of curari after prolonged action of nicotine. Further, the effects of solutions of curari of different percentage, when in both the percentage of curari is well below the equivalent ratio, differ but little, though the initial fall is slightly greater, the higher the curari percentage. The effect, small as it is at the beginning, soon diminishes, and after the first few minutes there is no constant difference in the rate of relaxation in the two solutions. There is a slightly greater rate of relaxation in the stronger solution of curari, but this is overborne by the intrinsic differences of the muscles. Fig. 28 shows the comparative effect of ·01 and of ·001 p.c. curari on muscles which had been placed in ·01 p.c. nicotine for 5 minutes.

From the results given in this section it follows that when curari is applied after nicotine, it antagonises the stimulating action of nicotine to a degree which depends on the relative concentration of the curarine and the nicotine, and that an absence of marked relaxation of the muscle in curari may be due to one or both of two causes, (1) the concentration of the curarine may be too small relatively to that of the nicotine, (2) the shortened state of the muscle may be due to contracture which is little or not at all affected by curarine.

4. THE EFFECT OF MIXTURES OF NICOTINE AND CURARI.

When the solutions of curari and nicotine are mixed before being poured on the muscle, the percentage of the curari must be very much greater than that of the nicotine in order to prevent contraction. Even when the percentage of curari is 50 times as great as that of the nicotine, a slight contraction is obtained in the rectus abdominis. With a mixture containing ·01 p.c. nicotine and ·1 p.c. curari, a prompt and considerable response is produced, though it is much less than that with ·01 p.c. nicotine alone. The contraction, however, soon diminishes. The less duration of contraction is seen with all mixtures, provided the ratio of the percentage of curari to that of nicotine is not too low. Two examples are given in Fig. 29. Presumably nicotine diffuses into the muscle more quickly than curari, so that some contraction is caused by nicotine and this is cut short by the following curari. The general effects are the same in the flexor carpi radialis[1] but the nicotine contraction, so far as my experiments go, is relatively greater, a result which may be attributed to the greater thickness of the muscle.

5. THE REGION OF ACTION OF NICOTINE AND CURARI.

The first point to be settled with regard to the point of action of nicotine and curari, pilocarpine and atropine, and certain other poisons, is whether they do or do not produce their effects by acting on the nerve endings, *i.e.* on the terminal branches of the nerve fibres. The answer to the question depends chiefly on whether the action of the poisons continues or is abolished when the nerve endings have degenerated.

---

[1] For the effect on the sartorius muscle cp. This *Journal*, XXXIX. p. 269. 1909.

In the case of mutually antagonistic poisons, practical certainty can be attained by observing after nerve degeneration the effect of the stimulating poison only, for it is practically certain that if the stimulating poison produces its effects by acting peripherally of the nerve ending, its effect cannot be prevented by any action the paralysing poison may have on nerve endings when these are present. I have shown that nicotine causes tonic contraction in denervated muscle, both in the fowl[1] and the frog[2] and the result in the fowl has been confirmed by Edmunds and Roth[3]. That I regard as satisfactory evidence that nicotine and curari in minimal doses act on something peripheral of the nerve endings.

Fig. 29. × 1⅓. Rectus abdominis. Effect of mixtures of nicotine and curari.

Further I have shown both in the frog and in the fowl that curari given before nicotine prevents a certain dose of nicotine, otherwise effective, from causing contraction. The action of curari in this case cannot be explained by an action on nerve endings.

---

[1] This *Journal*, XXXIII. p. 393. 1905.

[2] *Ibid.* XXXVII. p. 285. 1908.

[3] *Amer. Journ. Physiol.* XXIII. p. 28. 1908.

There remains the case when curari is given after nicotine. I found that a given dose of curari caused less relaxation of the nicotine contraction in a denervated than in a normal muscle. This decrease was emphasised by Edmunds and Roth and they were inclined to regard it as showing that curari did not antagonise the action of nicotine. Dixon[1] more definitely takes this view and considers that the results are in favour of the action of curari being on the nerve endings and not peripherally of them. We may then briefly review the facts of the case. Edmunds and Roth found that when a nicotine contraction had been obtained in a fowl's denervated gastrocnemius muscle, the subsequent injection of curari caused in one case good relaxation, in some cases slight relaxation, and in other cases no appreciable relaxation. He noted the form of the muscle curve for a short time only, and from the nature of the case was unable to compare the degree of contraction after curari was given with that of the normal muscle of the other side without curari. A trivial effect would escape notice in such conditions. Now we have seen above (p. 102) that the mere fact that the effect of curari is trivial is in itself no evidence that curari is not capable of antagonising strongly the stimulating action of nicotine, whether it does so or not depends on the ratio of the concentration of the curarine to that of the nicotine. Thus the inference of an absence of antagonism rests on a comparison of the degree of effect produced by a given ratio of curari to nicotine percentage in normal and in denervated muscles of different fowls. But beside the factor just mentioned there are two others which influence the degree of relaxation caused by curari, viz. the degree of responsiveness of the muscle to nicotine, and the extent of the contracture which it has produced. As the responsiveness of a muscle to nicotine increases, the antagonising influence of curari decreases. Further, an increase in responsiveness to nicotine involves the production of greater contracture by a given concentration of nicotine. Thus on both counts curari will cause less relaxation if a muscle becomes more responsive to nicotine. In the fowl I obtained some evidence that the denervated muscle was more responsive to nicotine than the normal one, and more conclusive evidence of this was obtained by Edmunds and Roth. It follows that the effect of a given amount of curari will almost certainly be less in a denervated than in a normal muscle, and that the extent of the decrease will vary with the extent of the increase of responsiveness to nicotine. In fact, the results obtained by Edmunds and Roth are such as were to be expected on the theory I have put forward. We may then conclude that the antagonism of nicotine and curari is the same in denervated as in normal muscles, and in consequence that curarine in all concentrations in which it has any effect acts peripherally of the nerve endings, and that therefore the phenomena give no basis for the assumption that curari acts on nerve endings. Since in the frog there is commonly no structure between the nerve and the muscle, I conclude that the action is direct on the muscle.

---

[1] *Proc. Roy. Soc. Med.* VI. p. 14. 1912.

Edmunds and Roth also made experiments on the antagonism of curari and physostigmine in denervated fowls' muscle. They found that it required about twice as much curari to cause relaxation of the tonic physostigmine contraction in denervated as in normal muscle. But sufficient curari in no case failed to cause relaxation. The difference in the effect of curari on the nicotine and the physostigmine contraction I take to be due to the muscle being much less responsive, as regards tonic contraction, to the latter than to the former.

The respective actions of pilocarpine and atropine on the salivary glands have a close resemblance with those of nicotine and curari on the muscle. Atropine and curari stop any stimulating effect which is going on, but do not restore the cells to their previous condition if any stimulating action has taken place.

I[1] have argued previously that the point of action of pilocarpine is the gland cell and not the nerve ending. Demoor[2] recently has also taken this view since he finds that perfusion with Ringer's fluid abolishes the effect of the chorda tympani without abolishing the effect of pilocarpine. The evidence would be more valid if it were shown that after perfusion the post-ganglionic and not simply the pre-ganglionic fibres of the chorda tympani were without effect.

Recently the view has been put forward[3] that the tone of striated muscle is due to impulses passing along sympathetic nerve fibres. If that is so, one might expect to find the effect of the sympathetic especially marked in the arm muscles of the frog and in the rectus abdominis. I have made a few experiments on the latter, but have not found that faradic stimulation of the rami communicantes of the spinal nerves supplying the muscle causes any contraction.

Nicotine causes muscular twitchings as well as tonic contraction. Both are set up in the neural region of the muscle. Both are prevented by a sufficient amount of curari. But we have seen above that the preventive effect of curari follows different laws in the two cases, so that caution must be exercised in drawing conclusions as to the twitchings from facts discovered with regard to the tonic contraction. I have earlier given some evidence that the point of action in the production and prevention of twitching is peripherally of the nerve endings, but the body of evidence for this is relatively small, and results have been described by other observers which *primâ facie* are not in harmony with this theory. The point of action of drugs which cause twitching I propose to investigate more fully at a later time.

---

[1] This *Journal*, XXXIII. p. 379. 1905.

[2] *Arch. Intern. de Physiol.* XII. p. 52. 1912.

[3] Cp. de Boer. *Folia Neuro-Biol.* VII. p. 378. 1913.

Note on the mode of action of nicotine and curari. We have seen that in the rectus abdominis muscle of the frog, the concentration of nicotine required to affect visibly the muscle outside the neural region is at least a thousand times that required to affect the neural region itself. This of course is only a particular example of the specific action of drugs. But the question of the mode of the specific action becomes much more definite if we can be certain as to the point of action of the poisons. In this paper I have endeavoured to clear up the doubts which have existed on the question as regards the tonic contraction, and to show that curari and nicotine produce their effects by an action on the neural region of the muscle fibre and not to an action on the nerve endings, so that the explanation of the specific action must depend upon a difference in the neural and non-neural region of the muscle fibres.

The theory that poisons having a specific action owe this property to their forming chemical compounds with certain tissues only is of very old date. The occurrence of mutual antagonism between pairs of alkaloids observed later was for a time contested, the action of the paralysing poison being considered irreversible. Examples of such antagonism were however given by Heidenhain for physostigmine and atropine, and by myself for pilocarpine and atropine. In order to account for this mutual antagonism I put forward the view that such antagonistic poisons formed chemical compounds with the same constituent in the tissues, that the compounds were reversible and that the amount of each compound formed depended on the relative masses (*i.e.* on the relative concentration) of the two poisons. The recent development of physical chemistry has suggested several ways in which the specific action of poisons might be accounted for, viz. by differences in permeability of the cell membrane, by differences in solvent power of the membrane, and by differences in surface area leading to differences in adsorption, but none of these at present give any satisfactory basis for an explanation of specific antagonism between particular pairs of alkaloids. But whilst advocating the chemical theory as being at present the one most in accordance with the facts, I do not consider it to be more than a tentative theory. What is now required is to find a means of testing the various possibilities. I am inclined to think that the rectus abdominis muscle is a favourable object for doing this. Some of the observations given in this paper have a bearing on the question, and these and others now in progress I propose to discuss in a later account.

SUMMARY.

The antagonism by curari of the fibrillar twitchings and of the tonic contraction caused by nicotine in the neural region of muscles follows different laws, for though curari reduces both effects in proportion to its percentage (*i.e.* in proportion to the concentration of its curarine), the abolition of the twitchings takes place with a definite percentage of curari for a considerable range of variation in the concentration of the nicotine, whilst the abolition of an immediate tonic contraction by curari is strictly dependent on the ratio of the percentage of curari to that of nicotine.

These conclusions are chiefly based on observations on twitchings in the sartorius muscle, and of tonic contraction in the rectus abdominis. In the sartorius, a percentage of curari which, with a given time of action, is just insufficient to prevent ·1 p.c. nicotine from causing twitchings is also insufficient to prevent ·005 p.c. nicotine from doing so. In the rectus abdominis the percentage of curari required to prevent immediate tonic contraction increases step by step with increase in the percentage of nicotine, thus when the curari is allowed to act for 15 minutes, solutions of ·00025 p.c., ·0025 p.c., ·025 p.c. and ·25 p.c. will (commonly) prevent immediate contraction being caused by ·0001 p.c., ·001 p.c., ·01 p.c. and ·1 p.c. nicotine respectively.

There is then a certain ratio between the percentage of curari and that of nicotine with which nicotine just fails to produce an immediate tonic contraction. This ratio may be called the equivalent percentage ratio. When the duration of the action of curari is 15 minutes, the equivalent percentage ratio is about $\frac{1}{50}$ to $\frac{1}{100}$ in the sartorius, $\frac{1}{2}$ in the flexor carpi radialis, and $2\frac{1}{2}$ in the rectus abdominis.

The action of curari is at its fastest at first, the rate of action then decreases. The higher the percentage of curari, the sooner a state of approximate equilibrium is reached. Satisfactory evidence of actual equilibrium in the reaction between curari and muscle was not however obtained.

When a solution of curari is insufficient to prevent nicotine from causing an immediate contraction, the rate of contraction caused will be the same whatever the absolute percentage of the two provided the ratio of the percentages is the same, and provided the contraction is confined to the neural region.

Curari does not appreciably antagonise the effect of nicotine on the non-neural portion of the muscle substance: the antagonism is confined to the special substance or mechanism of the neural region.

The effect of curari gradually disappears when the muscle is kept in Ringer's fluid, *i.e.* its action is reversible.

When a muscle is immersed in curari after having been immersed in nicotine, the extent of the relaxation produced depends on several factors; it is less the higher the percentage of the nicotine and, within certain limits, the longer the duration of its action; it is greater the higher the percentage of the curari. Other things being equal the extent of the relaxation depends on the relative concentration of curarine and nicotine. If the ratio of the percentage of curari to nicotine is below the equivalent percentage ratio the relaxation produced is trivial; and after a certain duration of action of the stronger solutions of nicotine, curari in higher percentage than the equivalent ratio also causes a trivial relaxation only. Thus curari antagonises the stimulating action of nicotine to a degree which depends upon the relative concentration of curari and nicotine, but it has no certain effect upon the contracture produced by nicotine.

An absence of distinct relaxation of the nicotine contraction when curari is given, such as occurred in some of Edmunds' and Roth's experiments on the denervated gastrocnemius of the fowl, does not show an absence of antagonism of curari for nicotine. The phenomena described are perfectly consistent with the phenomena which occur in undenervated muscles.

The experiments on the fowl as those in the frog show that both nicotine and curarine in minimal concentration affect something peripherally of the nerve endings, *i.e.* they affect a constituent of the muscles.

FURTHER OBSERVATIONS ON THE CONSTRICTING ACTION OF ADRENALINE ON THE CEREBRAL VESSELS. By CARL J. WIGGERS (*Cornell University Medical College, New York City*).

(*From the Physiological Laboratory of the University of Michigan, Ann Arbor, Michigan U.S.A.*)

In 1905 I(1) described some experiments from which I concluded that adrenaline causes a contraction of the blood vessels of the brain. The method adopted in these experiments consisted in decapitating a dog, tying the internal carotids intra-cranially anterior to the origin of the internal ophthalmic branch, and then inserting a cannula into the basilar artery. When this was done, repeated injections with various colour mixtures failed to show any injection of extra-cranial areas. Hence, the conclusion was drawn that, in a physiological sense, such a preparation was an "isolated brain."

The isolated brain was perfused at a constant temperature and pressure with a pulsating stream of Locke's solution, the fluid issuing from the cerebral veins dropping into the outflow recording apparatus.

The curve written was a straight oblique line if the outflow continued constant but became curved as soon as the outflow rate was changed. When adrenaline was introduced by a device which prevented even a momentary change in the viscosity of the perfusing fluid, its temperature or pressure, it was found that adrenaline in the strength of 1:500 decreased the outflow. In subsequent experiments (2) it was found that a reaction was obtained from 3 c.c. doses of solutions ranging in strength from 1:250 to 1:20,000. Weaker doses which still acted on other organs were found negative. Furthermore, the action of adrenaline was found to be abolished after apocodein while other drugs (among which may now be included pituitary extract) retained their action. The proof seemed, therefore, convincing that adrenaline constricted the cerebral vessels. In the same series of experiments another observation was made, the details of which have never been reported. It was found that if, after the onset of the adrenaline constriction, the perfusion pressure was raised centrally, the decrease in outflow which had just started was converted into an increase. This showed that the diminished outflow due to peripheral constriction was readily overpowered by a central rise in pressure (Fig. 1).

In 1910, Dixon and Halliburton(3) also described a method for perfusing an isolated brain. The method differed in that the entire brain was removed from the skull and perfused by Locke's solution under a lower pressure (200 mm. saline). The adrenaline solution was introduced by a hypodermic needle inserted through the tubing, a procedure that involves some elevation of the perfusion pressure in order to introduce the adrenaline. These investigators obtained only a slightly increased flow after adrenaline; a definite constriction, on the other hand, was never observed. In attempting to account for my positive results, Dixon and Halliburton(4) in a subsequent publication suggested they were due to constriction of extra-cerebral vessels, connected by anastomoses with those of the brain.

Fig. 1. Effect of adrenaline on the cerebral vessels when introduced without change in pressure. At *A* perfusion pressure just began to increase slightly and at *D* outflow began to decrease due to adrenaline. The perfusion pressure was then raised at *B* and the outflow at *E* increased beyond normal.

The basis for this criticism is probably found in the anatomical descriptions given by Ellenberger and Baum(5) who state that an anastomosis exists between a branch of the arteria corporis callosi and the posterior palatine and spheno-palatine arteries. Since in my experiments injection of the basilar artery with coloured fluids caused no colouration in the nasal cavity, I think the cerebral vessels were alone concerned. But it was obviously desirable to test the question on the brain removed from the skull, and this I have done in the manner described by Dixon and Halliburton except that the vessels were perfused with a pulsating stream. Fig. 2 shows a typical reaction upon introducing adrenaline with a hypodermic syringe. The perfusion pressure first slightly rose at *A*, which mechanically occasioned an increased outflow from *B* to *C*. This was followed by a decreased outflow at *D* and an elevation of the pressure at *E* due to peripheral constriction. Apparently the mechanical increase in outflow was obtained by Dixon and Halliburton but the real action was entirely missed. No explanation of the fact can be offered. The data of the different experiments are incorporated in the following table:

Fig. 2. Effect of adrenaline on the cerebral vessels—brain removed from skull. Letters referred to in text.

	Outflow before adrenaline	Outflow during adrenaline	Outflow after adrenaline	Perfusion pressure during adrenaline
1	27·0*	25·0	25·0	Rose 1 mm.
2	37·5	37·0	38·0	Rose 5 mm.
3	42·0	36·0	36·0	Rose 3 mm.
4	18·5	17·0	19·0	Rose 1 mm.
5	20·0	16·5	18·5	—
	18·5	15·0	17·0	—
6	41·1	39·0	42·0	—
7	36·0	36·0	35·0	No change.
8	23·4	23·3	23·3	,, ,,
9	14·5	10·1	13·0	Rose 3½ mm.
10	11·8	4·0	9·0	Rose 12 mm.
11	15·0	14·8	13·0	Rose 5 mm.
12	36·0	39·5	41·0	Fell 5 mm.
13	14·2	14·2	14·0	No change.

* All figures refer to mm. of rise in the outflow record during 30 second intervals.

The results show that out of 13 experiments upon the brain removed from the skull, adrenaline constricted the cerebral vessels in eight cases, in four no reaction occurred and a single one, which may have been due to an accidental rupture of vessels, an increased flow permanently followed.

REFERENCES.

(1) **Wiggers.** Amer. Journ. Physiol. xiv. p. 452. 1905.
(2) **Wiggers.** Ibid. xx. p. 206. 1907.
(3) **Dixon and Halliburton.** Quart. Journ. exp. Physiol. iii. p. 316. 1910.
(4) **Dixon and Halliburton.** This Journal, xlvii. p. 288. 1913.
(5) **Ellenberger and Baum.** Anat. des Hundes, p. 372. Berlin, 1891.

THE INFLUENCE OF CARBOHYDRATE AND FAT ON PROTEIN METABOLISM. III. THE EFFECT OF SODIUM SELENITE. BY E. P. CATHCART AND J. B. ORR.

(From the Physiological Department of the University of Glasgow.)

THE toxic effect of certain of the salts of selenium has long been known but so far no complete investigation of their influence on the animal metabolism has been attempted, A few years ago C. O. Jones (1) published a very interesting paper on the physiological effects of selenium compounds. He injected sodium selenite hypodermically and after the death of the animal extracted from the tissues the granular deposit observed by previous workers. This deposit he showed to be identical in every respect with the amorphous form of selenium obtained on reducing sodium selenite in the test tube. He found that the reduction took place if a solution of sodium selenite were allowed to act upon animal tissue in which enzyme action had been destroyed by heat and he concluded therefore that the reduction was a purely chemical action. He next examined the tissues for the particular constituent that effected the reduction and finally came to the conclusion that of substances of animal origin reduction could only be effected by glucose and a few allied sugars or bodies yielding these. This reduction could not be produced by organic matter from which these sugars were absent. To prove his point he carried out various forms of experiment; thus he administered hypodermically several sublethal doses of selenite of sodium to an animal which was then killed and its liver examined for glycogen. In a control animal the glycogen was abundant whereas in the injected animal it had almost completely disappeared. He inferred that the glycogen had been drawn upon to supply glucose for the reduction of the sodium selenite. As a result of these and other experiments Jones concluded, from apparently quite ample and well founded data, that sodium selenite injected hypodermically is carried by the blood stream to the tissues and is there reduced to selenium by glucose.

Assuming the validity of Jones's contention as to the intimate relationship between the reduction of sodium selenite and the glucose content of the tissues it was thought that a further study of the effect of these selenium salts might throw more light on intermediate carbohydrate metabolism and on the effect of disturbances of the same.

The animal employed in our experiments was an Airedale bitch of 12 kilos. weight. The bladder was emptied daily at 10 a.m. by catheter and the urine added to that collected in the receiver of the metabolism cage. The methods of estimation were total nitrogen by the Kjeldahl method, urea, ammonia, creatinine and creatine by Folin's methods, and amino-acids by the Henriques-Sörensen method. In all the experiments, with the exception of the first, the total amount of urine was made up to a constant volume before the estimations were made, as we have found in this laboratory that this procedure renders the analyses more accurate. The animal was always allowed to recover completely between the experiments. During each experiment the dog was kept on a constant fixed diet. After the diet had been given for from five to seven days, *i.e.* until the nitrogen output was steady for two or three days, a sublethal dose, viz. 2 c.c. of a 1 °/o solution of sodium selenite, was injected subcutaneously. The fixed diet was continued after the injection until examination of the urine showed that the output of the various nitrogenous constituents had returned to normal.

Throughout the series of experiments the protein content of the food was little altered but the amount of carbohydrate was varied from 33 to 204 grams per diem, while the fat content was varied inversely to maintain an approximately constant calorie value for the diet. Sodium chloride, 2 grams per diem, was added to each diet. The daily preparation of the food was carried out by J. B. O.

EXP. I. Diet. Oatmeal 40 grams, dry skimmed milk 30 grs., margarine 40 grs., tapioca 80 grs., containing approximately protein 28 grs., fat 44 grs. and carbohydrate 161 grs. with a total calorie intake of 1184 or about 99 Cal. per kilo.

Twenty minutes after the injection of the selenite the animal vomited, and continued to do so at intervals for about half an hour. The vomited matter consisted of saliva and mucus. Beyond this temporary sickness no ill effects were noted. The animal did not consume its full ration for the three following days but thereafter the appetite completely returned and the full amount was readily eaten.

TABLE I.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
3	970	2·63	1·96	·24	—	·362	·032	Full diet.
4	1099	2·71	2·00	·25	—	·367	·012	,,
5	1100	2·67	1·94	·24	—	·356	—	,,
7	Urine lost.							Injection.
8	240	4·02	3·29	·25	·03	·379	·153	⅓ diet.
9	349	2·98	2·29	·20	·01	·315	·148	⅓ diet.
10	560	2·57	2·03	·14	·02	·334	·045	½ diet.
11	460	2·12	1·65	·16	—	·343	—	Full diet.
12	700	1·94	?	·17	—	·357	—	,,

EXP. II. Diet. Oatmeal 70 grams, margarine 80 grs., and dry skimmed milk 30 grs., containing approximately protein 25 grs., fat 74 grs., and carbohydrate 64 grs., with a total calorie value of about 1053 Cal. or about 90 Cal. per kilo.

As in the previous experiment soon after the injection the animal vomited, but the vomiting persisted much longer. The animal appeared to be very lethargic and this condition of listlessness lasted during the following day and was still partially present on the second day after the injection. The animal refused its food on the day of the injection and on the two following days. On the fourth day it was quite normal and ate the full diet.

TABLE II.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	540	2·89	2·33	·19	—	·308	—	Full diet.
2	340	2·60	2·06	·18	·04	·318	·006	,,
3	602	2·98	2·27	·26	·01	·386	·028	,,
4	518	2·54	2·06	·20	·05	·346	·025	,,
5	390	2·24	—	·15	·06	·283	·061	,,
6	144	2·10	1·69	·13	·01	·368	·044	Fast.
7	520	2·55	2·06	·18	·05	·360	·053	Full diet.
8	525	2·55	1·74	·20	·04	·343	·054	,,
9	420	2·24	1·67	·20	·05	·291	·044	,,
10	340	4·27	3·59	·19	·06	·369	·157	Injection.
11	687	5·36	3·90	·38	·06	·302	·282	No food.
12	430	7·06	5·96	·41	·02	·352	·358	,,
13	200	3·37	2·67	·17	·09	·296	·036	Full diet.
14	330	2·32	1·84	·17	·06	·296	·016	,,

EXP. III. Diet. Oatmeal 100 grams, tapioca 80 grs., cane sugar 40 grs., dry skimmed milk 30 grs., containing approximately 25 grs. of protein, 27 grs. of fat and 189 grs. of carbohydrate with a total calorie value of 1128 or about 94 Cals. per kilo.

Twenty minutes after the injection the dog vomited mucus and saliva as in Exps. I and II but the vomiting was much less profuse. The animal was little upset and appeared quite lively and normal. No food was offered on the day of the injection. Next day it appeared quite normal but it only ate a portion of its food. On the following day the full diet was taken.

TABLE III.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	546	3·33	2·69	·32	·0003	·359	·029	Full diet.
2	650	2·63	2·18	·28	·09	·321	·029	,,
3	930	2·73	2·08	·31	—	·311	·027	,,
4	985	2·76	1·95	·29	—	·310	·027	,,
5	210	2·09	1·54	·13	·019	·288	·041	Fast.
6	840	2·45	1·98	·24	·027	·308	·015	Full diet.
7	690	2·08	1·55	·19	·029	·319	·011	,,
8	760	3·50	2·76	·23	·030	·339	·052	Injection.
9	420	4·65	3·48	·36	·105	·329	·121	Part food.
10	1000	3·67	2·52	·31	·036	·305	·011	,,
11	1000	2·86	2·15	·28	·020	·289	·005	Full diet.

Exp. IV. Diet. Oatmeal 30 grams, tapioca 100 grs., cane sugar 30 grs., dry skimmed milk 50 grs., and margarine 10 grs., containing about 23 grs. of protein, 12 grs. of fat and 204 grs. of carbohydrate with a calorie value of 1042 or about 87 Cals. per kilo.

Following the injection salivation was profuse but there was no vomiting. The dog appeared a little less lively than usual but was otherwise quite unaffected by the injection. No food was offered on the day of injection. Next day the animal appeared normal but ate only part of its food; on the day following it ate the full amount.

TABLE IV.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	698	2·84	1·85	·29	·02	·343	·026	Full diet.
2	690	2·71	1·79	·28	·05	·341	·004	,,
3	695	2·87	2·05	·29	—	·359	·016	,,
4	640	2·81	1·97	·30	—	·354	·012	,,
5	780	3·04	2·10	·29	—	·352	·011	,,
6	320	4·44	3·33	·26	—	·395	·055	Injection.
7	210	4·47	3·42	·28	—	·364	·035	⅓ food.
8	350	3·50	2·66	·29	—	·373	·032	Full diet.
9	630	3·22	?	·33	—	·357	—	,,

Exp. V. Diet. Oatmeal 30 grams, dry skimmed milk 30 grs., casein 9 grs., and margarine 100 grs., with about 25 grs. of protein, 85 grs. of fat and 34 grs. of carbohydrate, having a total calorie value of 1121, *i.e.* about 93 Cals. per kilo.

Within five minutes of the injection profuse salivation appeared and this was soon followed by vomiting of mucus, which was bile stained. The animal absolutely refused all food on the day of the injection and the following day but on the second day after it was induced with much coaxing to eat its full diet.

TABLE V.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	420	3·34	2·89	·20	·069	·384	—	Full diet.
2	375	3·23	2·83	·18	·075	·379	—	,,
3	410	3·03	2·10	·20	·052	·370	—	,,
4	255	2·94	2·12	·13	·063	·343	—	,,
5	Urine lost.							Injection.
6	275	4·42	3·32	·32	·081	·342	·298	No food.
7	220	5·24	3·91	·32	·098	·357	·189	Full diet.
8	510	4·68	3·52	·36	·125	·343	·053	,,
9	680	3·59	2·41	·33	·065	·331	·003	,,
10	340	3·31	?	·21	·069	·322	—	,,

EXP. VI. This was a short experiment to ascertain whether or no an acidosis resulted from the injection of the selenite.

Diet. Oatmeal 70 grams, margarine 60 grs., dry skimmed milk 30 grs., cane sugar 40 grs., containing approximately protein 25 grs., fat 52 grs. and carbohydrate 100 grs. with a total calorie content of 996 or about 83 Cals. per kilo.

Salivation began about three minutes after the injection. Vomiting of mucus and saliva occurred about half an hour later. Next day the dog ate about one third of its diet and on the following day two thirds.

TABLE VI.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Chlorides, gr.	Acetone + diacetic acid, gr.	Creatinine, gr.	Creatine, gr.	Remarks
3	680	3·76	·21	·068	?	·0044	·388	·051	Diet.
4	480	3·86	·25	·051	1·790	·0041	·377	·060	,,
5	385	3·86	·20	·074	1·340	·0047	·377	·060	,,
6	305	6·00	·35	·060	·017	·0054	·411	·227	Injection.
7	300	6·76	·39	·067	·028	·0081	·397	·240	⅓ diet.
8	265	5·21	·25	·051	·061	·0056	·385	·073	⅔ diet.

The acetone and the acetoacetic acid were estimated by means of the Messinger-Huppert method. In estimating the creatinine the readings were taken after the acetone and acetoacetic acid had been driven off by the method advocated by Graham and Poulton(3).

In order to ascertain whether selenium itself produced any effect on the metabolism a subcutaneous injection of a colloidal solution of selenium containing about ·030 gram of the metal was given to a dog on a fixed diet comparatively poor in carbohydrate. The urinary analysis showed no evidence of a disturbed metabolism nor were any of the other toxic symptoms, *i.e.* salivation, vomiting, present. The colloidal selenium was kindly given to us by Dr C. Walker of the Cancer Research Laboratory.

DISCUSSION OF THE RESULTS.

The general effects of the injection of the sodium selenite may be summarised as salivation, vomiting, anorexia and depression of vitality. The salivation which has not been noted by previous observers was always quite distinct and was the earliest symptom of the intoxication. In Exp. V it appeared within three minutes of the injection. The actual salivation was always preceded by a curious twitching of the nostrils and lips which suggested that there may have been some irritation of the nasal passages. It is possible that salivation may be due, as Jones suggests, to the irritation of the nerve endings of taste and smell by the methyl-selenide which is excreted by the lung and which causes the garlic like odour so constantly noted and commented upon.

Vomiting occurred in all of the experiments except IV, where the carbohydrate intake was highest. It is interesting to note that although the vomiting was such a prominent feature of the intoxication it seemed to cause no pain or even discomfort. No sooner was the actual act of vomiting over, except in experiment II, where depression was a marked feature, than the animal would take an active interest in its surroundings until the next attack suddenly came on. The vomiting was curiously intermittent. Associated with the vomiting was the marked anorexia. It is difficult to offer an adequate explanation of these phenomena but the following hypothesis may be of interest. The organism as a whole finds its existence threatened by the presence within it of a substance whose toxic influence can be nullified by glucose. There is therefore a call for the mobilisation of the maximum amount of glucose in the blood. This can be effected by increasing the sodium chloride content of the blood (Fischer (8)). It therefore diverts all its available chlorides to this end and in consequence the chlorine is withdrawn from the oxyntic cells of the stomach. Absence of HCl is a recognised accompaniment of, if indeed not a cause of, gastric disturbances associated with anorexia. That chlorides are utilised to increase the carbohydrate content of the blood is suggested by Jones who found a decreased excretion of chlorides lasting four or five days after a sublethal dose of the salt. In an experiment which we carried out to confirm this observation we found that the average excretion of chlorides in the urine for the three days preceding the injection was 1·98 grams per diem and the average for the three days following the injection was 0·035 gram. See also Exp. VI. This fall cannot possibly be accounted for by the abstention from food.

The severity of all these symptoms described above was in inverse proportion to the amount of carbohydrate previously ingested. Thus on the highest carbohydrate diet, viz. 204 grams per diem, there was no vomiting and the dog's health was but little disturbed, while on the lowest, viz. 34 grams, vomiting continued intermittently for 12 hours and for the two following days the animal was very lethargic.

Total nitrogen. As a result of the injection there is a marked rise in the output of nitrogen, indeed in every experiment the immediate increase is greater than fifty per cent. Whilst exact comparison is prevented owing to the varying amount of food taken on the days following the injection it will be noted that in general the increase is greater and continues longer where the preceding carbohydrate intake has been least.

As regards the source of this excreted nitrogen it can hardly be seriously maintained that the increased output is due to the drafting off of glucose to neutralise the toxic action of the twenty milligrams of sodium selenite injected and that as a result protein material is drawn upon to make up the deficiency. Of course part of the increased output on the days following the injection when no food was taken is probably accounted for on the grounds of energy requirement. In order to test this point in experiments II and III we interposed a day without food in the period preceding the injection but little or no rise in the output of nitrogen was observed. It is evident then that starvation does not play a very prominent part.

We believe that the increased excretion of nitrogen comes from a destructive disintegration of the protein molecule. It has been shown that the selenite is reduced to selenium within the cell, and that apparently the only substance capable of bringing about the reduction is carbohydrate. It is just possible therefore that the introduction of a small amount of selenite causes abstraction from the protoplasm of an equally small amount of carbohydrate, and that the loss of this carbohydrate results in a partial disintegration of the cell content with the subsequent excretion of the nitrogenous part.

Urea. The excretion of urea rises *pari passu* with the excretion of total nitrogen. Considered as percentage of the total nitrogen, taking the average of three days before injection and three days after, it will be seen from the following figures (Table VII) that, with the exception of experiment III, there is a rise in the percentage output of the urea following the injection. It would seem as if the metabolic processes concerned in the synthesis of urea were stimulated rather than depressed.

TABLE VII. *Output of urea in per cent. of the total nitrogen.*

Experiment—	I	II	III	IV	V
Average of 3 pre-days	73·8	73·9	76·5	70·5	71·2
Average of 3 post-days	79·7	80·4	74·1	75·9	75·0

Ammonia. It may be said, generally speaking, that the effect of the injection on the course of the output of ammonia is to cause a rise in the absolute amount excreted but a fall in the percentage amount considered in per cent. of the total nitrogen. Table VIII brings out a very interesting fact for which we cannot at present offer an adequate explanation, viz. that the higher the intake of carbohydrate in the food the higher is the output of the ammonia in the days preceding the injection.

Turning to the percentage output it is seen that here again the high carbohydrate intake is associated with a high percentage output in the days preceding the injection whereas after the injection no regularity in the output is to be detected. The question of the production of an acidosis will be considered in the discussion of the creatine output.

TABLE VIII.

Experiment—	IV	III	I	VI	II	V
Carbohydrate intake in grms. per diem	204	189	161	100	64	34
Average output of 3 pre-days:						
Absolute (in grms.) ...	·29	·29	·24	·22	·20	·17
Per cent.	10·1	10·8	9·0	5·7	7·9	5·5
Average output of 3 post-days:						
Absolute (in grms.) ...	·28	·30	·20	·33	·32	·33
Per cent.	6·8	7·5	6·2	5·4	5·7	7·0

Amino acids. The output of these bodies is not very marked; the only experiment in which a definite rise in the output took place was V. Here on the three days before the injection the average excretion was 0·063 gram per diem and on the three days following the injection 0·101 gram per diem. In experiment III there was a very transitory rise.

Creatinine. The injection of the selenite causes an immediate rise in the output of creatinine either on the day of the injection or the day following in all our experiments with the possible exception of V. Following this rise there tends to be a fall in the output on the day or two after this particularly noticeable in experiments II and V. The possible explanation of the fall below normal is that it is due to the presence of acetoacetic acid in these urines—the diets in both experiments were carbohydrate poor.

As our first five experiments were all carried out before the appearance of the observations on the influence of acetoacetic acid in the estimation of creatinine we controlled our results in experiment VI, in which the estimation of the creatinine was made *after* the removal of the acetone and acetoacetic acid. The only point which argues against such a conclusion is that in our observations (in another experiment, on a carbohydrate poor diet, the output of acetone and acetoacetic acid, as the result of the injection, rose from 7·7 mgrms. to 10·8 mgrms.) on the output of acetone and acetoacetic acid, after the injection of selenite, the rise, such as it was, only occurred on the day of the injection and the day after whereas the decrease in the creatinine output usually took place later.

When the output of creatinine is considered in percentage of the total nitrogen it is found, in every experiment, that the injection of the selenite produces a definite fall, as is clearly demonstrated in Table IX.

It will be noted that the fall is most marked in experiments II and V where the carbohydrate intake was lowest.

TABLE IX.

Experiment—	I	II	III	IV	V	VI
Average of 3 pre-days	5·05	5·38	4·31	4·54	4·40	3·70
Average of 3 post-days	4·09	2·35	3·10	3·43	2·70	2·48
Difference	·96	3·13	1·21	1·11	1·70	1·22

The results of these experiments certainly tend to subvert the view that creatinine is a direct derivative of creatine, formed from the latter substance by a method analogous to the formation of urea from ammonia. If the view, that some direct transformation occurred were correct, then one might expect a definite increase in the excretion of creatinine when a relatively enormous amount of creatine is being set free in the tissues and excreted in the urine (see also Table X) after each injection, unless the assumption also be made that some substance essential for this change is lacking as the result of the injections.

Creatine. Although we are inclined to agree with some recent workers who maintain that the available results are not sufficient to establish a direct causal relationship between carbohydrate and creatine metabolism, we think that the present series of experiments shows at least that alterations in the amount of available carbohydrate in the body does affect the output of creatine in a very marked fashion. One of us (E. P. C.(4)) has already stated elsewhere his belief that the output of creatine is not so much evidence of the disturbance of the creatine metabolism *per se* as that "the appearance of creatine under these conditions (*i.e.* lack of carbohydrate) is merely to be regarded as an index of faulty metabolism in general and perhaps more particularly of the non-occurrence of resynthesis."

Before discussing our results it is necessary to refer to some recent work on the estimation of creatine. Greenwald(5) pointed out last year that the presence of acetoacetic acid in the urine produced an alteration in the estimation of creatinine. The red brown colour of the Jaffe reaction is bleached, the degree of bleaching being proportionate to the amount of acetoacetic acid present in the urine. This statement has been confirmed recently by Graham and Poulton (*l.c.*), who maintain that the recorded observations on the output of creatine during carbohydrate starvation are purely fictitious, and that the difference between the colorimeter readings for preformed creatinine and total creatinine, which has been interpreted by many recent workers (Cathcart(6), Mendel and Rose(7), Wolf(8), Meyer and Fine(9), Krause and Cramer(10) and others) as being due to the presence of creatine, is in reality due to the presence of acetoacetic acid. We have carried out a number of observations on this point and find that the original observation of Greenwald is correct but that the contention of Graham and Poulton, that the acidosis alone accounts for the difference in readings, is not true. There is no doubt about the fact that when acetoacetic acid is added to urine the actual creatinine reading on the colorimeter is raised, *i.e.* the colorimeter reading does not give the true creatinine content in the presence of the acetoacetic acid, the result is therefore too low. This observation is of very considerable importance when acidosis is a marked feature of the urine which is being examined as for example in severe diabetes.

The question arises as to whether these observations invalidate the conclusion reached by one of us (E. P. C. (*l.c.*)) and since confirmed and extended by many other workers, that the output of creatine is associated in some way with disturbances in the carbohydrate metabolism. As we(11) have shown elsewhere, in the case of starvation, the output of creatine, which was found, cannot be accounted for by the amount of acetoacetic acid present in the urine. These observations were checked by the use of the method of Graham and Poulton (*l.c.*) for the removal of the acetoacetic acid, and still further controlled by the use of the diacetyl reagent of Walpole(12). We also found that creatine was actually excreted in the urine of a subject who was living solely on olive oil.

Still further evidence in support of the contention, that, if the course of carbohydrate be interfered with, creatine appears in the urine, is afforded by the output of creatine after the administration of phloridzin. In conjunction with Taylor one of us (E. P. C.(13)) found that, after the single injection of 0·75 grm. phloridzin, creatine appeared in the urine. It has been frequently demonstrated that it is extremely difficult to produce an acidosis in a dog, and further, Geelmuyden(14) and Baer(15) have both shown that a single small dose of phloridzin, such as we gave, does not produce an acidosis. This we have fully confirmed (*l.c.*). In the experiments of Wolf and Osterberg(16) large doses of phloridzin were repeatedly given and the output of acetoacetic acid was also estimated. They found that the creatine output preceded the appearance of the acetoacetic acid, and further, when the amount of the acid excreted is compared with the output of creatine, it is evident that the observed difference in readings between the preformed and the total creatinine could not be accounted for on the basis of an alteration due to the presence of the acetoacetic acid.

Then the recent experiments of McCollum and Hoagland(17) on carbohydrate starvation in the case of the pig yield the same result. They found that pigs fed on a carbohydrate diet excreted no creatine in the urine, but creatine was excreted on the last days of the experiment in which the diet consisted of fat and carbohydrate and was present throughout in the experiment in which fat alone was given as the foodstuff. At the same time they draw attention to the fact that it is difficult to produce an acidosis in the pig and they further state

TABLE X.

Experiment—	IV	III	I	VI	II	V
Amount of carbohydrate in grms. per diem	204	189	161	100	64	34
Amount of creatine excreted on the 3 days preceding the injection	·039	·083	·044	·171	·151	—
Amount of creatine excreted on the 2nd, 3rd and 4th days after the injection	·067	·137	·346	·540*	·676	·545
Increase in creatine output ...	·028	·054	·302	·369	·525	·545

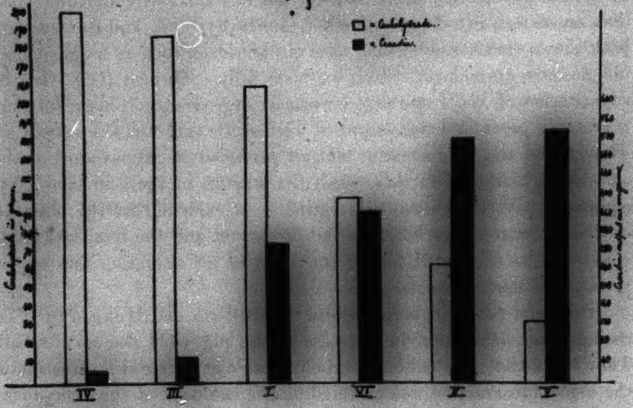
* In Exp. VI the excretion of creatine is taken over the 1st, 2nd, and 3rd days following the injecticn as this experiment was ended on the third day after the injection.

definitely that "acetone and diacetic acid were found only during the last few days of fat feeding."

In the present series of experiments, as will be seen from the tables I–VI and more particularly from Table X and Fig. 1, the effect of the injection of the sodium selenite is to cause a very definite output of creatine which varies in amount inversely with the amount of carbohydrate present in the diet.

The effect of the amount of carbohydrate in the diet on the output of creatine is most clearly shown in Fig. 1.

Fig. I



Incidentally it is interesting to note that in experiment V there was no excretion of creatine previous to the injection. The explanation of this may lie in the fact that the dog was allowed to get into first class condition before this experiment was carried out—it had a free open air life with abundant and varied diet. In order to test this the animal was again fed for some two months on an abundant mixed diet and allowed considerable freedom for exercise—the animal put on over a kilo. in weight—then given a carbohydrate low diet. No creatine was excreted for the first four days but on the fifth day a small amount appeared in the urine.

As was to be expected when the creatine output is considered in per cent. of the total nitrogen output it is found that following the injection there is always a very definite rise.

In view of the contention of **Hawk** (18) and his co-workers that creatine can be removed from muscle without of necessity causing complete disintegration of the protein molecule it is interesting to note the relation between the output of creatine and total nitrogen in the present series of experiments. We find that the ratio of creatine to total nitrogen in muscle tissue is approximately 1 : 10 and in practically all our experiments the creatine : total nitrogen ratio in the urine is greater than one to ten except in experiment V, where it is 1 : 9·4, and in experiment I. In this experiment the ratio for some unknown reason is only 1 : 5. This abnormal ratio results on the one hand from a very slight increase in the output of total nitrogen and on the other from an abnormally large output of creatine. Even experiment V, with its large output of creatine, does not in our opinion support **Hawk**. Here 0·545 gram of creatine was excreted, on the 2nd, 3rd and 4th days, which would represent the amount of creatine present in about 176 grams of muscle, as, according to the recent analyses of **Beker** (19), dog muscle contains about 0·31 °/₀ of creatine. If the output of total nitrogen is now considered it is found that the total increase (*i.e.* the total output of the three post-days less the total output of the three pre-days) as the result of the injection only amounts to some 5·14 grams. Assuming that the total nitrogen content of moist dog muscle tissue is about 3·3 °/₀ (the figure found in this laboratory by **G. D. Cathcart**), the output of nitrogen for the 176 grams of tissue should be about 5·8 grams. Thus in the experiment in which most creatine was excreted the output of creatine and the output of total nitrogen are practically in the proportion in which they exist in muscle tissue. Of course it is admitted that such a calculation as the above is only partially true, firstly because owing to the fact that the animal abstained from food on the 2nd day the actual extra output of total nitrogen must perhaps be regarded as 5·14 plus 4·42 = 9·56 grams, thus a ratio of 1 : 17·5, and secondly because it is highly improbable that muscle tissue is the only tissue attacked by the selenite and that therefore part of the nitrogen excreted will come from sources which are practically creatine free.

The question of a possible resynthesis having taken place does not in our opinion arise in the present series of experiments as the conditions for synthetic activity were far from favourable.

At present we are investigating the problem as to whether there may be some possible connection between the presence of creatine and the utilisation of carbohydrate or other source of energy in the muscle.

It is an interesting point that creatine is present in greatest amount about ·3 % in voluntary muscle where there are sudden demands for the liberation of energy, whereas in involuntary muscle it only amounts to about ·03 %; in cardiac muscle it takes an intermediate place about ·2 %.

CONCLUSIONS.

1. The injection of sodium selenite produces salivation, vomiting, anorexia, and lethargy.
2. The total nitrogen and urea outputs are raised as the result of the injection; this is most marked in the experiments with carbohydrate poor diets.
3. The ammonia output during the pre-days is highest with carbohydrate rich diets; on the days following the injection it is highest in the low carbohydrate experiments.
4. There is no marked acidosis following the injection as estimated by the output of ammonia, acetone and acetoacetic acid.
5. The injection of the selenite causes an immediate rise in the output of creatinine with possibly a subsequent fall below normal.
6. As a result of the injection there is a very definite rise in the output of creatine which cannot be explained by the presence of acetoacetic acid. The output of creatine varies inversely with the amount of carbohydrate in the food.
7. It may be said generally that the toxic effect of the selenite was most marked when the diet was poor in carbohydrate.

The expenses of this investigation were in greater part defrayed by a grant from the Carnegie Trust.

E. P. C. is responsible for the injections of the sodium selenite.

REFERENCES.

(1) **Jones.** Biochem. Jour. IV. 405. 1909.
(2) **Graham and Poulton.** Proc. Roy. Soc. B. LXXXVII. 205. 1914.
(3) **Fischer.** Arch. f. ges. Physiol. CIX. 1. 1905.
(4) **Cathcart.** Physiol. of Protein Metabolism, p. 116. London, 1912.
(5) **Greenwald.** Jour. Biol. Chem. XIV. 87. 1913.
(6) **Cathcart.** This Journal, XXXIX. 311. 1909.
(7) **Mendel and Rose.** Jour. Biol. Chem. X. 213. 1911.
(8) **Wolf.** Ibid. X. 473. 1911. Biochem. Zeit. XXXV. 329. 1911.
(9) **Meyer and Fine.** Jour. Biol. Chem. XV. 305. 1913.
(10) **Krause and Cramer.** Proc. Physiol. Soc. lxi. 1910 (This Journal, XL.).

(11) **Cathcart and Orr.** About to be Published in Proc. Physiol. Soc. for Mar. 14, 1914 (This Journal, XLVIII.).
(12) **Walpole.** This Journal, XLII. 301. 1911.
(13) **Cathcart and Taylor.** Ibid. XLI. 277. 1910.
(14) **Geelmuyden.** Zeit. f. physiol. Chem. XXVI. 381. 1898.
(15) **Baer.** Arch. f. exp. Path. u. Pharm. LI. 271. 1904.
(16) **Wolf and Osterberg.** Amer. Jour. Physiol. XXVII. 71. 1911.
(17) **McCollum and Hoagland.** Jour. Biol. Chem. XVI. 317. 1913.
(18) **Howe, Mattill and Hawk.** Ibid. XI. 103. 1912.
(19) **Beker.** Zeit. f. physiol. Chem. LXXXVII. 21. 1913.

THE CEREBRO-SPINAL FLUID. II. CEREBRO-SPINAL PRESSURE. BY W. E. DIXON AND W. D. HALLIBURTON.

(From the Physiological and Pharmacological Laboratories, King's College, London, and the Pharmacological Laboratory, Cambridge.)

CONTENTS.

PAGE
1. Experiments on the dead animal 130
2. Normal pressure 132
3. Effect of changes in arterial blood-pressure . . . 133
4. Passive effects of the cerebro-spinal pressure on cerebral venous pressure 139
5. Passive effects of cerebral venous pressure on cerebro-spinal pressure 140
6. The effect on cerebro-spinal pressure of substances which increase the cerebro-spinal secretion 142
7. Asphyxia 146
8. The effect of certain drugs 148

Introductory. The present paper is the outcome of investigations which followed on those recorded in the first paper of this series[1]. Our object was to examine the cerebro-spinal pressure under various experimental conditions. It will be referred to in subsequent pages as the C.S. pressure.

This pressure has been measured by a manometer by numerous observers, and it will be seen from the following figures[2] that it varies within considerable limits. In the majority of cases the observations were made in dogs in the horizontal position and the pressure was taken in the lower dorsal region. Cybulski 72–90 mm. of water, Adamkiewicz 80–100, Axel Key and Retzius 160–200, Bergmann 80–160, Quincke 40–60, Schultzen 52–100, Kronig 125–150, Parisot 100–150, Boveri 170–200.

Among these observers Axel Key and Retzius noted differences caused by respiration, their figures being 100–200 mm. of water during inspiration, and 250–275 during expiration. H. Falkenheim and B. Naunyn[1] give nearly the same estimate (160–200 in inspiration, and 250–275 in expiration). They also made extensive observations in dogs under ether anæsthesia, and under these circumstances place the normal C.S. pressure with the cannula inserted into the lumbar region, at 30–38 mm. of water, but state that when the carotid blood-pressure is high the C.S. pressure rises a variable amount and may reach 140 mm. of water (= 10·5 mm. Hg). They adopt the view that the fluid is a true secretion, and that it is formed in all probability by the choroid plexuses.

---

[1] This *Journal*, XLVII. p. 215. 1913.

[2] Taken from p. 85 of Mestrezat's book *Le liquide cephalo-rachidien normal et path.* Paris, A. Maline, 1912. See also Cavazzani, *Arch. ital. de Biologie*, p. 475. 1893.

They also state that changes of arterial blood-pressure are accompanied by similar changes in cerebro-spinal pressure; in strychnine convulsions the cerebro-spinal pressure rose but they incline to the view that this is mechanically produced and is not due to an increase of secretion. Compressing the veins leaving the central nervous system also raises the pressure in the cerebro-spinal fluid apparently by mechanical means. L. Hill[2] in his work on the cerebral circulation calls attention to the fact that variations in the cerebro-spinal pressure are closely related to alterations in venous pressure.

The present observations were made by connecting a cannula inserted into the subcerebellar cisterna, as described in Part I, with a manometer containing salt solution. The long arm of the manometer was connected by tubing to a Marey's tambour in which the rubber was so loose that within certain limits it recorded true volume changes. The tambour was calibrated before each experiment. Dogs anæsthetised with morphine and urethane were employed in all experiments.

It must be remembered that a manometer connected up in the manner described will transmit variations in pressure which may be due to many factors; increased secretion, or a block in the movement or absorption of the cerebro-spinal fluid, will obviously augment its pressure; but besides these, variations in the arterial blood-pressure transmitted through the brain substance, and in the venous pressure will also modify the result. Cerebral venous pressures were recorded by trephining over the torcula, removing the bone, and screwing in a brass tube connected with a manometer containing half saturated sodium sulphate or 10 °/₀ sodium citrate solution. A record was obtained by measuring the movements in the distal limb of the manometer. Indications of the changes in the cranial contents were also taken by a cup-shaped oncometer, one inch in diameter which when applied over a trephine hole of the same diameter could be made air tight by a clamp. Respiration as we have pointed out in Part I always exerts a profound effect on the rate of secretion so that in observations on pressure it is important that respiration should remain unchanged, and in all the present experiments efficient artificial respiration was performed throughout.

---

[1] *Arch. exp. Path. u. Pharm.* XXII. p. 261. 1887.

[2] *The Physiology and Pathology of the Cerebral Circulation.* London, 1896.

1. EXPERIMENTS ON THE DEAD ANIMAL.

Since the brain and its vessels and lymphatics are enclosed in a bony case it has generally been assumed that the brain and its vessels occupy the whole skull and that the brain being incompressible the total blood content is almost constant. This hypothesis is generally spoken of as the Monro-Kellie doctrine, and it has obtained general recognition. The advocates of this view suggest that the brain can increase its blood content only by turning out the cerebro-spinal fluid and that when this fluid has gone the brain comes in contact with the skull so that no further expansion of vessels can occur.

In order to test the validity of this hypothesis, experiments were performed in which the torcula pressure, arterial blood-pressure and C.S. pressure were recorded simultaneously, and sometimes the right auricle pressure was also recorded.

The first series of experiments were performed on the dead animal and in these the torcula pressure and C.S. pressure were recorded, a 20°/₀ solution of sodium citrate being used in both the manometer tubes. The death of the animal was caused by chloroform, hydrocyanic acid, or asphyxia. The artificial respiration was always kept up after death as the small oscillations in the pressures caused by this means served to facilitate movements of the fluid in the manometer tubes.

Although the circulation was stopped the blood remained fluid in the vessels, so that it was possible to test the effect of alterations of one pressure upon the other. Generally the two pressures were nearly equal, the venous being a little higher than the C.S. pressure. The venous pressure can be readily raised by injecting fluid into either the femoral vein or artery. Supposing that the initial artificial pressure in millimetres of 20°/₀ sodium citrate solution is between 50 and 60 mm. in each manometer, then raising the venous pressure to 130 mm. suddenly, causes only a gradual rise in the C.S. pressure of from 5 to 10 mm. which reaches its maximum after the venous pressure and falls only very slowly (see Fig. 1). A better method of increasing the venous pressure consists in compressing the abdomen with the hand, this raises venous pressure suddenly 50 or 60 mm. and when the hand is removed the fall is equally sudden (see Fig. 2); the C.S. pressure however rises very gradually 8 or 9 mm. but falls again suddenly when the increased venous pressure is removed. In these cases the initial C.S. pressure was low and a relatively large increase of venous pressure produced very little influence on the C.S. pressure.

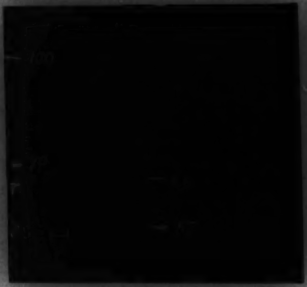


Fig. 1.

Fig. 2.

Fig. 1. Dog killed by chloroform. Torcula and c.s. pressures in 20 °/₀ sodium citrate solution. Shows the effect of injecting (at A) 45 c.c. normal saline solution into the femoral vein. Time = secs.

Fig. 2. Dog killed by chloroform. Torcula and c.s. pressures in 10 °/₀ citrate solution. Shows the effect of compressing the abdomen with the hand. Time = secs.

In another experiment the two pressures at the commencement were roughly equal and represented 120 mm. of 20 °/₀ sodium citrate solution. In this instance small changes of the venous pressure produced exactly similar changes of pressure in the C.S. fluid. In other words at this degree of distension in the dead animal the cerebral venous pressure exerts a decided passive influence on the C.S. pressure. It is clearly wrong then to imagine that the C.S. pressure must equal that of cerebral venous pressure. The ventricles of the brain hold an appreciable quantity of fluid and when this is at a relatively low pressure (such as 60 mm.) it is only with difficulty that the pressure can be increased passively by augmenting the venous pressure. If however the C.S. pressure is high to start with (100 mm. or more), so that the ventricles are distended with fluid, then this pressure is more sensitive to changes in venous pressure and within certain limits these two pressures may run parallel courses. Furthermore it cannot be argued that the C.S. pressure does not rise because the fluid escapes along the natural channels, since if it is raised artificially by the direct introduction of fluid it becomes equal to and runs parallel with the venous pressure for an indefinite period, and in this state of high tension, absorption of the fluid if it occurred at all in a dead animal should be greater than at the lower pressure.

2. NORMAL PRESSURE.

It has been pointed out already that after death the cerebral venous and C.S. pressures may or may not approximate to one another according to certain conditions. The same rule holds true during life. For example in one experiment on a dog the pressures at the commencement were; torcula 340, C.S. 190 mm. of 10 % sodium citrate solution. Ten minutes later the pressures measured; torcula 260, C.S. 170, and fifteen minutes later the venous pressure had fallen to 160 and the C.S. to 100. After the death of the animal one hour from the first record the two pressures were approximately equal at 65 mm. This is no exceptional condition. In other experiments the two pressures have remained roughly parallel throughout, unless experimental procedures have been adopted to alter them, but the venous was always higher than the C.S. pressure. Perhaps the most remarkable feature of the C.S. pressure viewed from the physical side is the fact that relatively small circulatory changes as determined by the general arterial and venous pressures may cause large changes in the cerebral venous pressure but have very little effect on the C.S. pressure.

The normal pressure of the cerebro-spinal fluid is variable as previous observers have noted, but in most of the present experiments in which the animals were anæsthetised in the manner mentioned and in which sodium sulphate was used in the manometer tubes the normal pressure was nearer the lower than the higher limits of the figures already given, a rough average being 40–70 mm. of salt solution.

In the case of one dog the pressures which were taken with similar manometers using a 10 % citrate solution varied during the experiment as follows:

Observations at 5-minute intervals.

Arterial blood-pressure	140—120—110—140—150—170—165—180—140—100—120—110—115—115—120—120 mm. Hg.
Venous pressure	350—294—280—260—280—260—300—415—225—230—220—230—260—330—240—255 mm. citrate solution.
C.S. pressure	95— 25— 30— 35— 55— 25— 80— 65— 65— 75— 70—60— 55— 50— 80— 90 mm. citrate solution.

These figures are typical of numerous other experiments. The venous and arterial pressures in the above experiment are higher than normal, but the C.S. figures are typical. It is quite evident that no simple relationship can be obtained between any of these, which may vary quite independently of one another. One law always holds, however, under all ordinary conditions; the arterial blood-pressure is always higher than the venous pressure, and the venous pressure than the C.S. pressure.

The use of citrate solution in the manometer tubes is more satisfactory than sulphate as regards prevention of clotting, but it has disadvantages. If much of the solution reaches the general circulation, the deficiency of ionic calcium in the body renders the central nervous system hyperactive so that the arterial pressure, venous pressure and C.S. pressure are all high and natural respiration is very difficult to paralyse with anæsthetics. In the present experiments half saturated solution of sodium sulphate was used in the torcula manometer and normal saline in the C.S. manometer unless it is stated otherwise.

3. Effect of changes in arterial blood-pressure.

(*a*) *Hæmorrhage.* The simplest method adopted for lowering blood-pressure was bleeding; in one typical experiment during natural respiration 100 c.c. of blood was removed from the femoral artery of a dog, after two minutes a second 100 c.c. and still later a third 100 c.c. The blood-pressure and cerebro-spinal pressure fell together at each withdrawal of blood but the first bleeding produced much the greatest effect. The animal was then bled to death, but no further fall in C.S. pressure was registered, and at the moment of death when respiration ceased, the pressure rose 20 mm. In another experiment the effect of drawing off from the femoral artery 200 c.c. of blood was to cause a fall in the C.S. pressure of 30 mm. saline. The curves of systemic blood-pressure, cerebral venous pressure, and C.S. pressure showed in their broad features similar outlines but the relative amount of fall was very much greater in the case of the arterial and

Fig. 8. C.S. and torcula pressures in 10% citrate solution. Shows the effect of withdrawing 75 c.c. blood from the femoral artery. Time =2 secs.

venous pressures. At the end of one experiment (Fig. 3) 75 c.c. of blood was withdrawn, this caused a fall in torcula pressure from 380 to 230 mm. of 10 °/$_\circ$ citrate solution and a fall in c.s pressure from 160 to 135 mm. of the same solution. In these cases the fall in c.s. pressure should be regarded therefore as passive, the direct result of the altered vascular conditions.

(*b*) *Compressing blood vessels.* Compression of the aorta is a simple and uncomplicated way of raising the arterial pressure in the cerebral region. The aorta had a ligature passed round it just below the diaphragm and drawn through the abdominal wound which was then closed: by putting some tension on this ligature the lumen of the artery was blocked completely. In one dog on which this experiment was tried the original pressure of the cerebro-spinal fluid was equal to 50 mm. of salt solution: the aorta was compressed for brief periods of 10 or 20 seconds and the c.s. pressure rose in successive compressions, 8·5 and 15 mm. (see Fig. 4). On ceasing the compression the pressure rapidly fell to the normal, and then more slowly until a subnormal pressure was reached: the fall below normal after compression usually approximated to the rise above normal during the compression. In the

Fig. 4. c.s. pressure in normal saline and blood-pressure in femoral artery. Shows the effect of compressing the abdominal aorta. The blood-pressure in the carotid artery rose 45 mm. Hg. Time = secs.

experiment quoted the falls of pressure following on the rises of 8·5 and 15 mm. were 7·3 and 22 mm. saline respectively. The "brain volume" showed a corresponding slight increase and the venous pressure in the brain measured by taking the pressure in the torcula a much more considerable effect. Any such rise in the carotid blood-pressure then is associated with a corresponding rise in (1) venous pressure (relatively large), (2) in c.s. pressure (relatively small), and (3) increase in the brain volume. If the c.s. fluid is free to escape from an open cannula during the rise in pressure, then after the first slight gush due to the increased vascular volume in the cranium, secretion either ceases or goes on at its normal rate. In other words such vascular changes do not augment the c.s. secretion. The rise in pressure is entirely the result of the altered vascular conditions. This being so it is natural to expect that the pressure would fall after a time even whilst the altered vascular conditions obtain, on the assumption that there is a free outlet for the escape of fluid. That the outlet is free is easily shown by puncturing the theca lower down and placing a second cannula in the mid-dorsal region; if 20 c.c. of saline are injected into this cannula the c.s. pressure as measured by the cannula in the subcerebellar cisterna rises but the extra fluid is soon absorbed and the pressure falls again to normal. So long, however, as the aorta is clamped the c.s. pressure remains up within the limits of experiments which extended for some two or three minutes. On two occasions the pressure was continued for a quarter of an hour; in these instances the pressure gradually fell but came to rest before the normal was reached. These facts suggest that the increased c.s. pressure is due to the altered vascular conditions and that these conditions either prevent the free absorption or facilitate the secretion of fluid and so keep the pressure high. The fact, which is well recognised clinically, may be pointed out here that sudden rises in blood-pressure in man such as may occur for example in acute nephritis are associated with augmented pressure in the c.s. fluid. This subject will receive further consideration in a later paper.

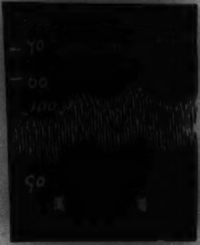


Fig. 5. c.s. pressure and blood-pressure in femoral artery. Shows the effect of compressing both carotids. Time = secs.

(c) *Clamping both carotid arteries* is another method of interfering with the circulation so as to induce purely passive effects on the c.s.

fluid. The effect is shown by a relatively great fall in venous pressure, a relatively small fall in C.S. pressure 5–7 mm. of saline (Fig. 5), and a diminution of "brain volume." If the two vertebral arteries are tied first, clamping the carotids exerts a more decided action but even this procedure only causes a fall of 10–15 mm. in the C.S. pressure.

(*d*) *Stimulation of the vagus and sympathetic nerves.* Excitation of the central end of the vagus is yet another means of causing a considerable rise of both arterial and cerebral venous pressures; the C.S. pressure rises also to a small extent. The character of the rise is very similar to that which is caused by compressing the aorta and may be regarded as the result of the altered vascular conditions. In these experiments it was noted that the C.S. pressure often begins to fall and even reaches normal during a stimulation lasting only 40 or 60 seconds. A subnormal pressure then invariably occurs before the constant normal level is reached. Stimulation of the peripheral end of the cervical sympathetic which had been carefully separated from

Fig. 6. C.S. pressure and arterial blood-pressure. Shows the effect of exciting the right vagus with the secondary coil at 10 cms. Time = secs.

the vagus never produced changes in the C.S. pressure nor in the torcula pressure.

Excitation of the peripheral end of the vagus causes both immediate and delayed effects. The immediate effects are, of course, a fall in carotid blood-pressure; less blood passes to the brain and therefore the brain volume diminishes. The cerebral venous pressure is dependent upon the systemic arterial blood-pressure and the right auricular pressure: the former drops almost to zero whilst the latter rises very considerably. The torcula pressure therefore varies according to these two factors. Usually whilst the stimulus lasts (say for 30 seconds) a small fall in the torcula pressure is recorded but when the stimulation ceases the arterial pressure rises before the veins can efficiently empty themselves; therefore a very considerable increase in the torcula pressure may occur at this time (Fig. 8). The C.S. pressure would not be influenced directly by cardiac inhibition but as both the arterial and venous cerebral pressures fall it might be expected that some passive diminution would occur, and this is frequently the case (see Fig. 6). Thus in one experiment a short vagal stimulation lasting 20 seconds, and which produced decided cardiac slowing and fall of blood-pressure equal to 50 mm. of mercury, also caused a fall in torcula pressure equal to 40 mm. of sodium sulphate solution and a fall of C.S. pressure equal to 15 mm. of saline.

When the stimulation ceased the C.S. pressure rose immediately 35 mm. that is 20 mm. above the normal and then slowly regained the normal condition.

We have pointed out in Part I that stoppage of the circulation tends to increase, at least for two or three minutes, the secretion of C.S. fluid so that during a long stimulation of the vagus (after the initial passive fall) the C.S. pressure begins to rise very slowly at first, but more rapidly later, and when the stimulation of the vagus is stopped and the blood-pressure recovers, the C.S. pressure rises considerably above the normal. After cessation of the stimulus and especially if the carotid pressure rises much above the normal the brain volume and torcula pressure will be increased correspondingly but the rise of C.S. pressure is greater than can be accounted for by any such passive effect. Furthermore in some experiments in which vagal stimulation caused complete cardiac inhibition and in which the secretion of the fluid was especially active it was found that the cardiac inhibition (which produced a fall of carotid pressure to zero and a correspondingly large fall in torcula pressure) was unable to reduce passively the C.S. pressure; this commenced to rise immediately gaining acceleration with each second of the stimulation (Fig. 7): for example during one experiment in which electrical stimulation of the right vagus with the secondary coil at 10 cms. caused a rise of 20 mm. of saline in the first 10 seconds, the continuation of the same stimulus caused a rise of 50 mm. in the next 20 seconds. This effect then must be due to increased secretion of fluid or to some failure of the fluid to escape; the former must be the more important factor since if the fluid is free to escape from an open cannula cardiac inhibition may cause a considerable increase in the rate of flow.

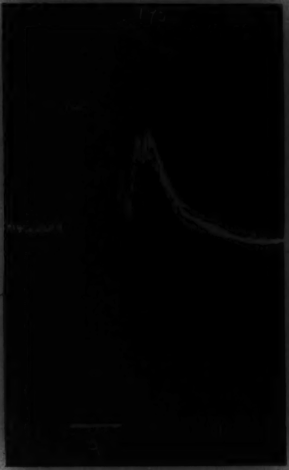


Fig. 7.

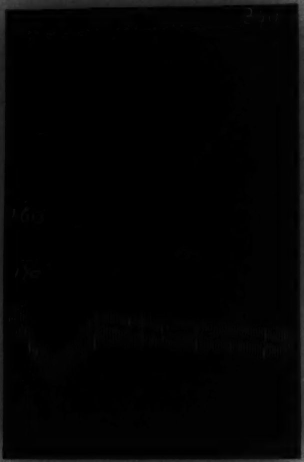


Fig. 8.

Fig. 7. C.S. pressure and arterial blood-pressure. Shows the effect of stimulating at 'A' the right peripheral vagus with the secondary coil at 10 cms. The torcula pressure is not recorded but it fell during the period of inhibition, but subsequently rose 40 mm. of sodium sulphate solution. Time = 5 secs.

Fig. 8. Torcula (upper line). C.S. and arterial pressures. Shows the effect of exciting the left vagus with the secondary coil at 15 cms. Time = secs.

From this it is clear that stimulation of the peripheral end of the vagus may cause (1) when the fall of blood-pressure is small a slight passive diminution of the C.S. pressure without any subsequent rise even though the torcula pressure rises greatly (Fig. 8); (2) an initial passive fall in pressure followed by a rise which commences whilst the arterial and venous pressures are still low (Fig. 6); or (3) an immediate rise in c.s. pressure which may be regarded as being caused by increased secretion due to deficient oxygenation or collection of carbon dioxide in the brain (Fig. 7).

4. PASSIVE EFFECTS OF THE C.S. PRESSURE ON CEREBRAL VENOUS PRESSURE.

It becomes necessary now to examine how far these two pressures may influence one another by passive means. If the two cannulæ in the torcula and subcerebellar cisterna respectively are connected to two manometers, it is an easy matter to raise the pressure of the c.s. fluid and note the effect on the venous pressure. Such a rise always produces a passive increase in the cerebral venous pressure but not to the extent of the additional increase of c.s. pressure. Thus if the two pressures to start with are approximately 70 and 140 mm. then raising the c.s. pressure 100 mm saline increases the venous pressure only by about 40 mm. (Fig. 9): the rise is steep but not so steep as the fall when the pressure is removed If the pressure is increased to 200 mm. the rise in venous pressure is 75 mm. These figures are given for a rise of pressure lasting 45 seconds. At the end of this period the venous pressure was still actively rising and if the increased c.s. pressure is allowed to continue till no further change occurs in the venous pressure it is invariably found to be higher than the c.s. pressure, provided that the latter pressure has not been raised artificially more than a few hundred millimetres. It is evident therefore from the direct experiment that alterations in the c.s. pressure exert a marked passive action

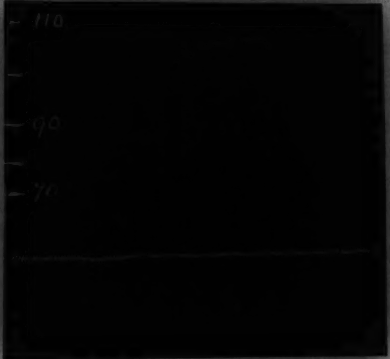


Fig. 9. Torcula and arterial blood-pressure. Shows the effect of raising the c.s. pressure 100 mm. of saline solution. Time = secs.

on the venous pressure, and provided no greater pressures than 100 or 200 mm. of saline are employed no material alteration occurs in the arterial blood-pressure. This factor requires careful consideration when investigating the action of chemical substances, as those which cause an increase of C.S. pressure from augmented secretion must cause secondarily some, though not a corresponding, increase in the cerebral venous pressure. It might be imagined that when the C.S. pressure exceeds the venous pressure as in the experiment quoted that so much pressure would be exerted as to partly block the venous circulation. This is not however the case. Measurements of the blood flow from the internal jugular vein during these conditions show no appreciable diminution in blood flow. If the C.S. pressure is raised still higher so that a serious block might be placed on the outflow of blood from the brain then in order to compensate for this condition the arterial blood-pressure also rises, but a consideration of these cases we defer to Part III. In all these pressure effects it is desirable to bear in mind that the vessels connecting the arteries to the veins are not under effective vaso-motor control, so that any rise of arterial blood-pressure is associated necessarily with a rise in venous pressure.

5. PASSIVE EFFECTS OF CHANGES IN CEREBRAL VENOUS PRESSURE ON THE C.S. PRESSURE.

This of course cannot be investigated by injecting fluid into the torcula since the fluid immediately drains away and the pressure remains at the same height. The injection of 50 or even 100 c.c. of saline into the torcula cannula within the space of 20 seconds exerts no decided influence on the C.S. pressure. The obvious method of increasing the cerebral venous pressure is to clamp the veins from the head as near to the right auricle as possible but without opening the thorax. Ligatures can be placed round these veins and the wound stitched up so that by gentle tension on the ligatures the lumina of the vessels are occluded. This procedure raises the pressure in both the torcula and cerebro-spinal fluid (see Fig. 10). By this means in three experiments the following figures were obtained:

	Exp. 1		Exp. 2		Exp. 3	
Torcula pressure	190	215	170	215	190	280
C.S. pressure	150	155	150	160	155	180

The first figure in each case represents the normal pressure and the second the maximum height to which the pressure rose as a result of compression. It will be noted that in each instance the rise in C.S. pressure is only about one quarter that which occurs in the torcula. It may be pointed out also that in all these experiments the two series of pressures are very high. When they are lower, as is more usual, compressing the veins has little or no effect on the C.S. pressure. It follows therefore that the effect of C.S. pressure on cerebral venous pressure is considerably more important than the effect of cerebral venous pressure on C.S. pressure and that when the two pressures are low the passive effect of increasing the venous pressure is negligible.

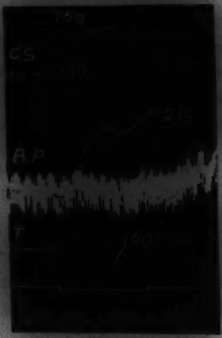


Fig. 10. C.S. pressure, arterial blood-pressure and torcula pressure. Shows the effect of compressing the veins in the neck. Time = 2 secs.

Compression of the abdomen with the hands is another means by which venous pressure may be raised but in this instance the experiment is not clean, for arterial blood-pressure may be materially altered, and the activity of the heart is changed, so that the significance of figures obtained by this method is not great. In three experiments the following figures were noted:

	Exp. 1		Exp. 2		Exp. 3	
Torcula pressure	245	260	240	255	120	130
C.S. pressure	180	190	80	90	60	65

The first figure represents the normal pressure and the second that obtained during compression. When the compression was stopped the venous pressure occasionally showed a secondary rise of from 50 to 100 mm. but this had relatively little effect on the C.S. pressure. The first effect is due to passive venous congestion brought about in the same way as ligaturing the veins, the second and delayed rise in venous pressure we think is due to increased cardiac output; at all events in these cases an increased cardiac output occurs and it is remarkable that this has very little or no effect on the C.S. pressure.

The torcula pressure may be raised by two principal means (1) passively, due to back pressure, (2) actively, due to increased cardiac output. When the pressure rises as a result of the former method it affects passively the C.S. pressure to a greater extent than when it rises as the result of increased cardiac output.

If the arterial and venous pressures are lowered, as for example by bleeding, the drop in the C.S. pressure is relatively small. It is certain then that the C.S. pressure is not greatly affected by circulatory changes except perhaps when it happens to be very high; it is an independent pressure and the factors determining its height are dependent on the rate of secretion and the rate of absorption of the fluid.

6. THE EFFECT ON CEREBRO-SPINAL PRESSURE OF SUBSTANCES WHICH INCREASE THE CEREBRO-SPINAL SECRETION.

The conclusion stated in the preceding sentence, that C.S. pressure is an independent secretory pressure (although it may be influenced passively by circulatory changes) received abundant confirmation when we proceeded to investigate the effects upon it which are exercised by substances which our previous work had shown us increase the flow of the fluid. Under this head, observations have been specially directed to three substances, choroid extract, chloroform and carbonic acid gas. The first was administered by intravenous injection and the two latter by inhalation.

The effect upon arterial blood-pressure of the first two is to cause a fall: it might therefore be anticipated that were this circulatory factor the only one concerned a simultaneous fall of C.S. pressure would occur; but in all cases the C.S. pressure rises and it is obvious that the change in arterial pressure cannot produce that effect. It is, however, important to remember that all three substances increase the cerebral venous pressure, and so it is necessary to prove that this vascular change is not the cause of the rise in C.S. pressure.

(a) *Choroid extract.* We have already shown that extracts of the choroid gland increase the rate at which the C.S. fluid is excreted from an open cannula: the injection of this substance should therefore increase the C.S. pressure, and as a matter of fact the injection of 5 c.c. of a 1 °/₀ solution into a vein always causes an increase in the pressure. Usually the rise commences at once and is synchronous with the fall in arterial blood-pressure (Fig. 11), but sometimes a latent period of half a minute or longer may follow the injection, and during this time the pressure either remains constant or may fall slightly. The rise in C.S. pressure is generally about 20 or 30 mm. of saline, and lasts two or three minutes. The venous pressure usually falls a trifle immediately after the injection probably on account of the diminished output from the left ventricle but when the arterial blood-pressure rises the cerebral venous pressure rises more rapidly and may rise as much as 20 mm. of sulphate solution above the normal. This rise in the torcula pressure is associated with a small rise in the right auricular pressure, and similar effects can be obtained with numerous other substances (such as the choline group) which slightly depress the heart and cause a fall in systemic blood-pressure.

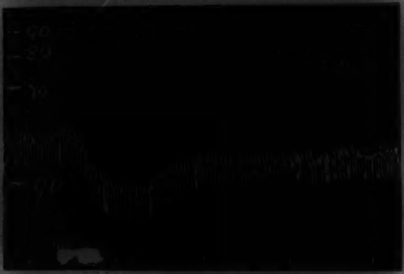


Fig. 11. C.S. pressure and arterial blood-pressure. Shows effect of injecting 5 c.c. 1 °/₀ solution of boiled choroid extract into femoral vein. Time=30 secs.

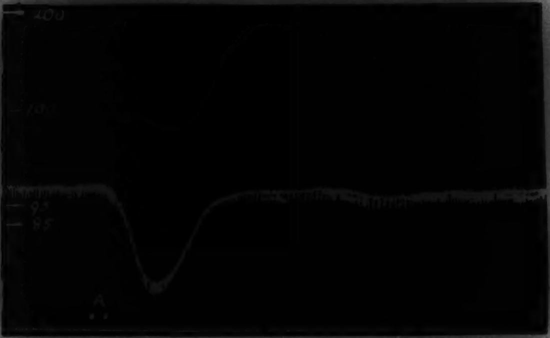


Fig. 12. Torcula pressure and arterial blood-pressure. Shows effect of injecting at 'A' 5 c.c. 1 °/₀ solution of boiled choroid extract into femoral vein. Time=secs.

The curves of C.S. and venous pressures do not run parallel with one another, as the former generally rises immediately after injection, whilst the latter rises only when the arterial blood-pressure commences to rise (Fig. 12). It may be noted that in one instance in which no change in the arterial blood-pressure was registered as the result of an injection, the rise in C.S. pressure was nevertheless well marked. The increase in C.S. pressure is therefore specific and due to increased secretion of fluid.

(*b*) *Anæsthetics and hypnotics.* Chloroform inhalation generally produces a much more decided increase in C.S. pressure than choroid extract: a rise of 40 mm. is no uncommon effect. The cause of this must be due to one of three conditions. It may be that some cause is damming up the secretion so that its natural outlet is blocked: this however cannot be the explanation since we have shown already in Part I that chloroform causes a greatly increased flow of secretion. The effect might be caused by altered circulatory conditions pressing upon the channels in which the C.S. fluid lies. The arterial blood-pressure certainly cannot effect this since it always falls whilst the C.S. pressure rises. The venous pressure does not always behave in the same way during the inhalation of chloroform. Usually after relatively small doses the torcula pressure rises and this rise occurs soon after the arterial blood-pressure begins to fall but neither its height nor length corresponds with the fall in arterial blood-pressure. This rise must be due to the increased right auricular pressure since it occurs at a time when the output of blood through the carotids is diminished. Larger doses of chloroform

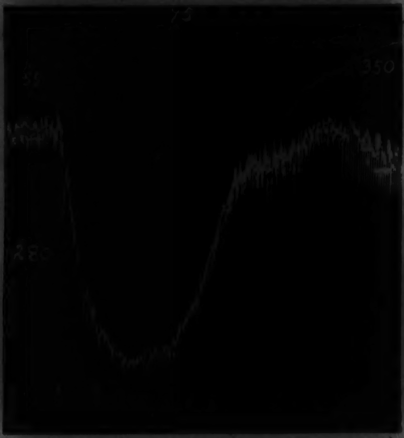


Fig. 18. C.S. pressure (top line), arterial pressure, and torcula pressure. Effect of chloroform inhalation (between the arrows). Time=2 secs.

which lower the arterial blood-pressure to a great extent still cause the immediate rise in C.S. pressure, but now the torcula pressure also falls and may remain subnormal often until the arterial blood-pressure has reached its normal height. The venous pressure now begins to rise rapidly often going up 100 mm. of sulphate solution, but even this sudden rise exerts no effect on the C.S. pressure (see Fig. 13). The venous pressure is dependent on two factors (1) cardiac output and (2) resistance on the right side of the heart, that is right auricular pressure: the fall which occurs first is due to diminution in the cardiac output and the second effect (the rise) to increase in right auricular pressure. From this it is certain that the C.S. pressure is completely independent of cerebral venous pressure so that only one explanation remains which can account for the phenomenon, namely that it is due to an entirely independent secretory pressure. Other anæsthetics and hypnotics produce similar types of effect to those described. Urethane injected slowly into the circulation may cause an increase in the C.S. pressure without influencing either the systemic blood-pressure or torcula pressure. Ether given by inhalation also causes very little fall in arterial blood-pressure or rise in venous, and alcohol often none, and yet both of these cause some increase in the C.S. pressure though not to the same extent as chloroform.

(c) *Carbon dioxide.* It has been shown already that an excess of carbonic acid in the blood increases the rate of flow of the C.S. fluid from an open cannula placed in the cisterna, and this at a time when the blood-pressure is not materially raised. It might be expected then that this gas when inhaled should increase the C.S. pressure. This expectation has been confirmed. The gas has been administered by the respiration pump freely diluted with air in amounts varying from 2 to 20 %; though usually 3 or 4 % produced a decided effect yet 10 % produced a better result in the short period during which it was administered. In these experiments the animal must be anæsthetised sufficiently deeply to prevent spontaneous respiratory efforts. The effect begins a few seconds after the inhalation commences; the C.S. pressure rises steeply whilst for a time the venous pressure remains unchanged. Then the venous pressure also slightly rises and its contour closely follows the curve of the C.S. pressure except that the degree of change is much smaller (see Fig. 14). When the inhalation ceases the C.S. pressure falls, but more slowly than it rises and takes perhaps two or three times as long to reach the normal as it takes to rise to its maximum height. The rise in venous pressure is insignificant and can be explained by the passive effect of the rise in C.S. pressure and perhaps also by the rise in arterial pressure when this occurs. The reasons for this view are that the changes in venous pressure always follow accurately those of the C.S. pressure and because the pressure in the right auricle often remains unchanged at a time when the torcula pressure is maximal. The increased C.S. pressure then must be regarded as a pure secretory rise and the changes in venous pressure as partly at least a passive effect the direct result of this.

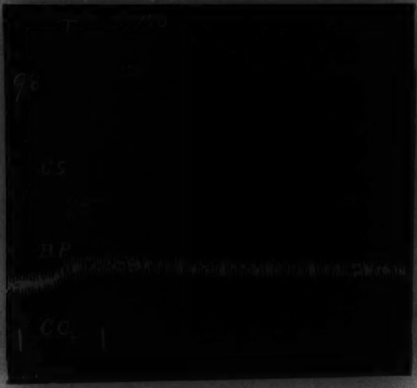


Fig. 14. Shows effect of inhaling 5 °/₀ CO_2 in the air. Time = 5 secs. (*T* = torcula pressure; C.S. = C.S. pressure; B.P. = arterial pressure.)

7. ASPHYXIA.

The changes in the C.S. pressure during asphyxia were studied in animals so deeply anæsthetised that the medulla had lost its respiratory action. At the instant artificial respiration is stopped, in typical experiments the torcula pressure first falls a trifle, it then gradually rises with the rise in arterial pressure and continues to rise when the arterial pressure begins to fall, rising much more steeply when the heart fails (see Fig. 15). These changes closely correspond with the changes of pressure in the right auricle though the sudden rise in torcula pressure during cardiac failure is more marked than the corresponding increase in pressure which occurs in the right auricle.

The C.S. pressure rises almost immediately, slowly at first but more rapidly later and often goes up as much as 100 mm. of saline (Fig. 15). This pressure commences to fall before the arterial blood-pressure has reached its maximum height and long before there is any decided increase in venous pressure. During the sudden rise in torcula and general venous pressures the C.S. pressure is rapidly falling and when the venous pressure is not far from its maximum the C.S. pressure may be down far below its normal.

Fig. 15. Asphyxia. Time=secs.

Clearly then the changes in C.S. pressure cannot be accounted for by vascular conditions, but they can be explained on the assumption that carbonic acid which collects in the blood during asphyxia excites the secretory epithelium to activity and produces a secretory pressure which is entirely independent of all vascular changes. The general appearance of the curve is the same as that which is obtained when an excess of carbonic acid is inspired at a time when a plentiful supply of oxygen is reaching the blood. The final fall in the C.S. pressure must be referred to the circulatory failure, and no very decided fall occurs until the heart has nearly ceased beating.

If the animal has been receiving other substances which influence secretory activity and if the asphyxia is performed at the end of an experiment devoted to other objects the typical effect of asphyxia is often lacking, that is the sudden rise in C.S. pressure whilst the arterial and venous pressures are almost constant. In these instances the C.S. pressure may closely follow the curve of the arterial blood-pressure, gradually rising to a maximum at the time when blood-pressure is maximal and falling during cardiac failure (Fig. 16).

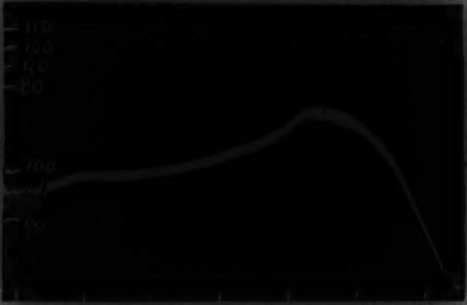


Fig. 16. C.S. pressure and arterial blood-pressure. Asphyxia. Time=30 secs.

8. THE EFFECT OF CERTAIN DRUGS.

Three typical drugs have been taken to illustrate the effect of vascular changes on the C.S. pressure, adrenaline to represent a typical vaso-constrictor, amyl nitrite to represent a vaso-dilator, and pilocarpine to represent the group of drugs which induce cardiac inhibition.

(*a*) *Adrenaline.* The rise of arterial pressure produced by this substance is always accompanied by a rise in the fluid of the manometer connected with the cranio-spinal space (see Fig. 17). This begins with the rise in systemic pressure and follows it until a maximum is reached but after this the two curves no longer run parallel. The C.S. pressure tends to fall much more rapidly than the blood-pressure so that when the blood-pressure is normal the C.S. pressure may register 20 or 30 mm. of saline below normal. The rise in C.S. pressure is apparently mechanical, the direct result of the filling of the cerebral arteries and veins and the transmission of the increased pressure through the C.S. fluid to the manometer.

The pressor action is associated with increased torcula pressure and increased "brain volume," but the increase in these pressures is not comparable with that which obtains during cardiac failure, asphyxia, or after chloroform inhalation: these two curves run a course roughly parallel with the systemic arterial pressure and must be regarded as the direct outcome of it. It may even be stated in general terms that all drugs which increase blood-pressure cause a corresponding increase in the cerebral venous pressure, and as may be seen if a cerebral vein is opened, a greatly increased flow of blood occurs, which is roughly proportional to the rise of blood-pressure, an additional proof if one were needed that the cerebral vessels possess no efficient vaso-motor nerves. The C.S. pressure then does not follow the blood-pressure except during the rise. It is not uncommon to notice in the atropinised dog that the C.S. pressure may be increased 40 mm. of saline and in the normal dog 15 to 20 mm. after the injection of 0·1 mgrm. adrenaline. During the increase in systemic blood-pressure the C.S. fluid is under increased pressure and it is probable that this leads to a temporary increase in the rate of the flow of the fluid to its natural outlets from the brain, and therefore as soon as the blood-pressure begins to fall a subnormal pressure is established. It appears then that adrenaline affects the C.S. fluid only indirectly through the vascular system. Pituitary extract, nicotine, tyramine and other pressor bases act similarly to adrenaline.

Fig. 17. Shows the effect of injecting into the jugular vein 1 c.c. 1 in 20,000 adrenaline. Atropine had been given previously. Time = 5 secs.

(*b*) *Amyl nitrite.* This drug given in moderate doses, such as the inhalation of three or four drops, increases the secretion of C.S. fluid[1], and hence should tend to increase C.S. pressure. A rise in C.S. pressure is the rule (see Fig. 18); it comes on immediately the arterial pressure falls and assumes the normal position again about the same time as the arterial blood-pressure. This effect is often associated with a general rise in venous pressure including torcula pressure. Frequently the C.S. pressure falls a little at first with the initial drop in arterial blood-pressure but soon recovers and shows an augmented pressure even while the blood-pressure is at its lowest. Now amyl nitrite by lowering systemic resistance causes the output of blood from the heart to be increased. This factor and the small rise which occurs in the right auricular pressure are sufficient to account for the effect on torcula pressure; and the two curves representing cardiac output as measured by the cardiometer and torcula pressure run a roughly parallel course. The "brain volume" also increases whilst the C.S. pressure is high. The rise in C.S. pressure cannot be regarded as secondary to alterations in the venous pressure because it begins and reaches its maximum before the rise in venous pressure, often five to ten seconds before. In those cases when the increased cardiac output is decided the torcula pressure may rise considerably, 100 to 150 mm., but it was noticed that this large increase coming on when the C.S. pressure is already maximal is without any appreciable effect on this pressure. Furthermore it is by no means

---

[1] In Part I we attribute the increase of cerebro-spinal flow produced by this drug as due to interference with the due oxygenation of the blood.

Fig. 18. c.s. pressure, arterial pressure, and torcula pressure (top line). Shows the effect of two inhalations of amyl nitrite at 'A' and 'B.' Time = 5 secs.

very uncommon to note that the venous pressure is unaltered by nitrites in the doses under consideration, or even that it falls whilst the C.S. pressure is actively rising. Thus in one experiment the torcula pressure fell 35 mm. of sulphate solution whilst the C.S. pressure increased 10 mm. and in another the torcula pressure fell 10 mm. whilst the C.S. rose 30 mm. The rise in C.S. pressure has certainly all the characteristics of an independent factor. It might of course be argued that the rise in torcula pressure is the passive effect of the rise in C.S. pressure, and no doubt this may be one of the determining factors in the rise and sometimes may be the sole one, but in such cases the rise would be very slight.

(c) *Pilocarpine*, as has been noted in Part I, has a double action on the C.S. secretion of which the more important factor is its action upon respiration. It has in addition a true secretory effect. If this alkaloid is injected into an anæsthetised dog with natural respiration it causes a great increase of C.S. pressure, 70 to 100 mm. saline, which falls again when atropine is administered in spite of the sudden and great rise in arterial blood-pressure. This rise in pressure is associated with a large and corresponding rise in the general venous pressure and in torcula pressure and these three curves run a more or less parallel course and all three slowly assume the normal condition when the cardiac inhibition is removed by atropine. If the animal is subjected to forcible artificial respiration sufficiently strong to counterbalance any moderate degree of bronchial constriction the effect on the C.S. pressure is not quite the same. Here a gradual rise in C.S. pressure occurs of about 20 to 30 mm. saline, which is further increased, though only temporarily, to double or treble that amount when atropine is given on account of the sudden increase of the vascular cerebral volume. The torcula and general venous pressures on the contrary are not influenced by the artificial respiration, they rise in just the same way as before and fall when the atropine is administered. That is to say atropine causes an immediate and great rise in C.S. pressure and an immediate and great fall in torcula pressure (Fig. 19).

It is noticeable that the general venous pressure and torcula pressure rise immediately and steeply; the C.S. pressure, however, falls, the degree and amount of fall depending on the extent of the venous rise. Thus if the rise in venous pressure is great, 50 to 80 mm., the fall of C.S. pressure is slight and is followed by a moderate rise in pressure apparently passive, and largely dependent on the rise of venous pressure, because if the rise in venous pressure is slight the rise in C.S. pressure may not occur for a minute or even longer. In the latter instance, this delayed rise is clearly not passive; it is entirely independent of venous pressure and should be regarded as a secretory rise, and we have shown that pilocarpine has such an action.

If whilst the heart is beating very slowly atropine is given the arterial blood-pressure bounds up to normal and the general venous and torcula pressures rapidly drop to normal. The C.S. pressure on the contrary rapidly and decisively rises to two or three times the height it was when the torcula pressure was maximal. This sudden rise in C.S. pressure cannot then be due to passive venous effects, indeed it rises in spite of the rapid venous fall. And arterial passive effects we have pointed out already have relatively little effect on C.S. pressure. The effect must be ascribed to a secretory pressure. During the

Fig. 19. Shows the effect of injecting into the femoral vein 20 mgms. pilocarpine nitrate (A) followed by 8 mgms. of atropine (B). Time = 5 secs.

Fig. 20. Torcula (top line), C.S. and arterial pressures. Shows the effect of injecting 5 mgms. pilocarpine nitrate into femoral vein (A) followed by 1 mgm. atropine (B). Time = 30 secs.

inhibition of the heart the cerebral circulation is almost at a standstill and oxygenation is deficient: the return of the circulation causes activity in the choroid gland with the resulting increase of pressure. Small doses of pilocarpine, arecoline, choline and other members of this group which produce a depressor effect without any marked effect on the rate of the heart, and many of which owe their depressor action largely to vaso-dilatation, show another picture. In these cases the injection of the drug causes the three curves, torcula, C.S. and blood-pressures, to assume a similar type; all three fall together, but the fall in C.S. pressure is insignificant compared with that of the torcula (Fig. 20). When the blood-pressure rises after atropine the torcula and venous pressures also rise and assume a level considerably above the normal.

CONCLUSIONS.

This investigation has shown that the cranial contents cannot any longer be regarded as a fixed quantity without the power of expanding or contracting in volume. The C.S. pressure is influenced passively to a small extent by changes in the arterial and venous pressures but such alterations are insignificant compared with the independent changes in pressure which occur as the result of secretory activity. Of all the conditions which influence the C.S. secretion we attach the most importance to deficiency of oxygen or excess of carbon dioxide in the blood.

We are indebted to the Government Grant Committee of the Royal Society and to the Science Committee of the British Medical Association for grants towards the expenses of this research.

VASO-MOTOR NERVES IN THE LUNGS.

By ENID M. TRIBE.

(From the Physiological Laboratory of the London (Royal Free Hospital) School of Medicine for Women.)

THE question as to whether vaso-motor nerves are, or are not, present in the lungs has been a subject of debate for many years, and has been investigated in various ways by many workers.

In 1894 Bradford and Dean[1] showed that a rise of pressure in the pulmonary artery occurs on stimulation of the 3rd, 4th and 5th thoracic nerves. Brodie and Dixon[2], in 1904, introduced a method of artificial perfusion of the lung vessels, thereby eliminating possible complications from effects on the heart, but they were unable to confirm the results of Bradford and Dean with nerve stimulation. They also investigated the action of adrenalin upon the pulmonary vessels, but obtained no evidence that it had any constrictor effect, a very slight dilatation only being occasionally produced. Plumier[3], by simultaneously registering pressures in the carotid artery, pulmonary artery and left auricle, showed that large doses of adrenalin, such as 2 mgms., produced vaso-constriction in the lungs, and he suggested that the negative results of Brodie and Dixon might be due to the use of amounts too small to be effective.

In 1909 the subject was again attacked by Wiggers[4], by means of the lung perfused with saline solution. He also obtained constriction with adrenalin, with amounts much less than those used by Plumier, obtaining constriction with doses of 0·2 mgm., but not with smaller amounts. Shortly after, Argyll Campbell[5] showed that "Hemisine" causes constriction in the lung vessels, but very much less than in the vessels of other organs.

---

[1] Bradford and Dean. This *Journal*, xvi. p. 34. 1894.

[2] Brodie and Dixon. *Ibid.* xxx. p. 476. 1904.

[3] Plumier. *Journ. de Physiol. et de Path.* vi. p. 655. 1904.

[4] Wiggers. *Journ. of Pharm. and Exp. Therap.* i. p. 348. 1909.

[5] Argyll Campbell. *Quar. Journ. of Exp. Physiol.* iv. p. 1. 1910.

Langlois and **Desbouis**[1], in 1912, stated that large doses of adrenalin—about 1 mgm.—undoubtedly cause vaso-constriction, but that 0·05 mgm. causes vaso-dilatation.

Recent work by **Pick** and **Baehr**[2] again supports the view of **Brodie** and **Dixon** as to the absence of vaso-constrictor fibres. They were able to show no change in the outflow from guinea-pig's lung perfused with Tyrode's solution after the addition of 0·01 mgm. adrenalin per litre of the perfusing fluid. **Starling** and **Fühner**[3], using a "heart-lung" preparation, obtained evidence of slight constriction in the lung arterioles on injection of even such small quantities of adrenalin as 0·01 mgm.

In the present research, which is a continuation of the work on which a preliminary note was published some time ago[4], the effect of adrenalin upon the pulmonary circulation was investigated by means of the method introduced by **Brodie** and **Dixon**, namely the artificial perfusion of the lung *in situ* with the defibrinated blood of the animal, the circulation of the blood being maintained by means of a small pump and glass valves. The outflow from the pulmonary veins is collected (through a cannula tied into the left auricle) in a glass receiver (*A*) (Fig. 1), from which the blood is sucked by each stroke of the pump and forced into a second reservoir (*B*), immersed in a water bath at body temperature. By means of a cylinder of compressed oxygen and a mercury valve and manometer, the blood is here oxygenated and forced onwards under a known pressure, which can be read off on the manometer and adjusted to the required height by the mercury valve. After passing through a small tube lightly packed with glass wool to act as a filter, the blood reaches the lungs by means of a cannula tied in the pulmonary artery.

To obtain a graphic record of variations in the outflow from the lungs, the receiver (*A*) is connected with a sensitive volume recorder. With each thrust of the pump, therefore, the recording lever falls, as blood is sucked out from the receiver, but rises again as blood flows in from the vein. The thrust of the pump is adjusted so that the amount sucked out with each movement is exactly balanced by the inflow from the vein before the next thrust. As long as the flow is constant, therefore, the general direction of the tracing will

[1] **Langlois et Desbouis.** *Soc. Biol.* LXXII. p. 674. 1912.

[2] **Pick und Baehr.** *Arch. f. exp. Path. u. Pharm.* LXXIV. p. 65. 1913.

[3] **Starling and Fühner.** This *Journal*, XLVII. p. 286. 1913.

[4] **Tribe.** *Proc. Physiol. Soc.* LXX. 1912. (This *Journal*, XLV.)

be horizontal. Reduction of the flow, as with vaso-constriction, will cause a fall of the recording lever, while vaso-dilatation, giving an increased flow, will be shown by a rise in the tracing.

When a change in the flow occurs, the blood accumulates in one of the reservoirs, *A* or *B*, and diminishes in the other. By means of a tube (*D*) connecting *A* and *B* and fitted with two clamps and a syringe, blood can be drawn from *A* and returned to *B* or *vice versa* according as dilatation or constriction has occurred. This sudden increase or decrease in the amount of blood in *A* will of course be recorded by a vertical movement of the lever.

In its main features, therefore, the method was exactly that of Brodie and Dixon, but alterations were made in one or two details.

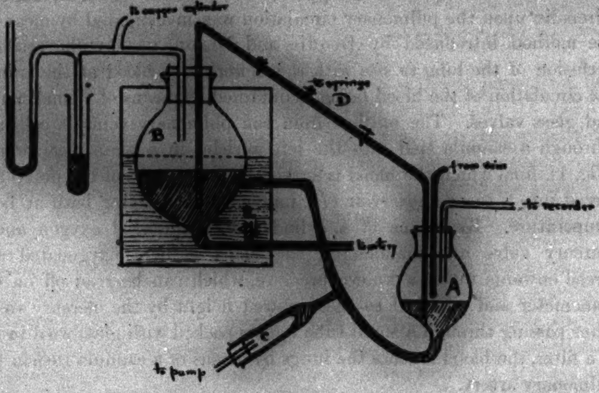


Fig. 1.

To prevent the blood coming in actual contact with the pump, a fairly stout rubber finger stall (*c*) was inserted between the pump and the blood, the rubber bag and the pump being filled with dilute glycerine. Rhythmic contraction and expansion of the bag therefore occurs with each stroke of the pump, thus acting as a heart to drive the blood forward. It was also found that the lung vessels are exceedingly sensitive even to small changes of temperature, and two modifications were introduced to lessen the possibility of variation of temperature.

Throughout the experiment the animal was kept immersed in a bath of ·9 °/₀ saline solution at 37 ° C.; and injections of the solutions under investigation were made through a side tube (*E*) under the surface of the water surrounding the blood reservoir (*B*), so that by keeping the syringe containing the solution in position in the water bath for several minutes beforehand, the injection could be made at exactly the same temperature as that of the perfusing blood.

The actual experiments were performed as follows:

The animal, usually a cat, was pithed, artificial respiration performed and the blood collected through a cannula in the carotid artery, defibrinated and filtered through glass wool. Cannulæ were then tied into the pulmonary artery and the left auricle, and the ventricles of the heart tied tightly round with a thick ligature. In some cases, especially if the supply of blood was small, the lung on the right side was completely cut out of the perfusion by tying round the base of the lung on that side, but no difference in the results of the experiments was noted in these cases. If the supply of blood was very small, the amount of perfusing fluid was increased by the addition of Ringer's solution or of Tyrode's solution, but since dilution of the blood leads quickly to œdema, this was only done when the blood was too small in amount to fill the apparatus.

The blood was then poured into the receiver (*A*) and the pump started, the animal placed in the saline bath, the artery and auricle connected to the perfusion apparatus, and perfusion commenced after adjustment of the pressure. In the case of dogs a pressure of from 15 to 20 mm. Hg was used, for cats generally from 10 to 15 mm. and for smaller animals preferably under 10 mm. It was found most important that the pressures used should not be above those mentioned; if in cat's lung, for instance, a pressure higher than 20 mm. Hg is used, the pulmonary artery becomes distended, the lung tissue œdematous and mottled, and it is found difficult to produce effects with small doses of drugs. This is in agreement with results recently obtained by Wiggers[1], who finds that the normal pressure in the pulmonary artery varies from 10 to 27 mm. Hg.

The animals generally used were cats, but experiments were also performed on dogs, rabbits, rats, guinea-pigs and a ferret. In the case of the smaller animals since the amount of blood obtainable from a single animal was insufficient to fill the apparatus, the blood from a second animal was often used in addition, in preference to dilution with saline solution. With rabbits some difficulty was found in perfusing with defibrinated blood, since the serum appears to have a marked constrictor action upon the pulmonary vessels making it very difficult to obtain an adequate flow; but this difficulty was finally overcome by the use of hirudinised blood.

---

[1] Wiggers. *Amer. Journ. Physiol.* XXXIII. p. 1. 1914.

Various preparations of adrenalin were used, but the one most commonly employed was Parke Davis's crystalline preparation of the base "adrenalin," which was dissolved immediately before injection in defibrinated blood. The solution was diluted to the required amount and 2 c.c. injected.

Fig. 2.

Fig. 3.

At body temperature and at normal pressures of perfusion, the primary effect of adrenalin is a marked diminution of venous outflow, indicating vaso-constriction. The amount and duration of the constriction obtained vary with the strength of the solution injected.

In cats, if 2 mgms. of adrenalin are injected, a sudden very marked and prolonged constriction is obtained (Fig. 2), while with smaller doses, for instance, ·2 mgm., a decided constriction, lasting from one to three minutes, and changing to a rapidly increasing dilatation is produced (Fig. 3). The injection of ·02 mgm. causes only a very brief constriction followed almost immediately by a dilatation (Fig. 4).

Fig. 4.

Fig. 5.

With still smaller doses the constrictor effect is entirely lost, but the dilator effect still remains. Thus with an injection of ·002 mgm. only dilatation is produced (Fig. 5). The minimum amount capable of producing dilatation in these perfusion experiments is ·00002 mgm. but the smallest dose with which preliminary constriction has been obtained is ·01 mgm.

It thus appears that we are here dealing with two opposing actions of adrenalin, constriction being produced only by comparatively strong doses and dilatation by much smaller amounts. In the other animals used, similar constriction was obtained, but the after dilatation was much less marked or altogether absent (Figs. 6 and 7); in no case was such a sudden change from constriction to dilatation obtained, as is so often seen in the cat's lung.

Fig. 6.

Fig. 7.

Other preparations of adrenalin, including Burroughs and Wellcome's "Hemisine" and extracts of fresh and of dried suprarenal glands, were found also to produce constriction on injection (Fig. 8). The investigation of the action of Parke Davis's solution of adrenalin hydrochloride (·1% in saline) led to some very interesting results. This preparation was the one mainly employed by Brodie and Dixon; and experiments performed in the earlier part of this research appeared entirely to confirm their results, viz. that adrenalin hydrochloride, injected even up to the amount of 2 mgms., produces only dilatation. It therefore seemed that the actions of the base adrenalin and of adrenalin hydrochloride were precisely opposite, since the injection of 2 mgms. of adrenalin invariably caused prolonged constriction. Among other possible causes of this difference in action were the presence of chloretone and the acidity of reaction of the adrenalin hydrochloride solution. Chloretone, however, in the concentration in which it is present in this preparation, has only a slight dilator action upon the lung vessels, much less than that shown by the chloretone and adrenalin hydrochloride together. The question of the acidity was next investigated, but injection of small amounts of HCl had practically no dilator effect, and in some cases even caused slight constriction. Freshly made adrenalin hydrochloride, obtained just before use by addition of a drop of HCl to a solution of the base, was found to cause constriction as readily as the base itself. Another possibility was that the reduction of the viscosity of the blood by the injection of the saline used as the solvent in the commercial solution might be the cause of the absence of the constrictor effect, but the dilatation produced by the injection of a few c.c. of saline was found to be slight and of very short duration, so that again it seemed some other cause must be assigned.

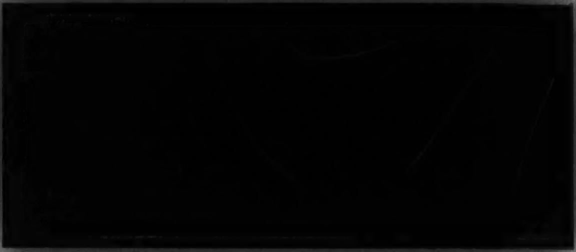


Fig. 8.

Further experiments showed later that the important factors in determining whether or no constriction is produced by adrenalin are the temperature and pressure at which the perfusion is performed. The earlier experiments were made with a pressure of about 40 mm. Hg, but at this stage it was realised that such a pressure was abnormally high, and all future perfusions were performed with a pressure of from 10 to 15 mm. Hg only. It was then found with this lower and normal pressure, that the immediate effect even of the commercial solution of adrenalin hydrochloride is constriction followed by dilatation more or less rapidly according to the amount injected (Fig. 9). This preparation does not produce such a marked constriction as the base adrenalin or as the freshly made adrenalin hydrochloride, and the constrictor effect is much sooner lost on dilution; but it is clear that the actions of all preparations are essentially the same, though, owing probably to the dilator action of the chloretone and the saline in the commercial hydrochloride solution, and possibly also to gradual destruction of the adrenalin on keeping, the constrictor action of the latter preparation is much weaker than that of other forms. With high pressures it seems probable that the comparatively weak constricting action of the hydrochloride solution is unable to overcome the passive dilatation due to the excessive pressure, and thus only the dilator effect of the adrenalin is obtained. As a confirmation of this, an experiment was performed with a perfusion pressure of 50 mm. Hg, and it was found that at that pressure adrenalin hydrochloride produced no constriction (Fig. 10) and even 0·2 mgm. of the base adrenalin produced dilatation only.

Fig. 9.

The question of temperature was also shown to be most important. It was found that at room temperature a reversal of effect often occurred, dilatation being produced even with strong doses of adrenalin (1 to 2 mgms.). This effect was most easily shown on the saline perfused lung with its comparatively rapid flow, since with a blood perfusion fall of temperature caused such a marked diminution of flow that the thrust of the pump could not be made sufficiently small for a horizontal tracing to be obtained. In the saline organ at body temperature, or even several degrees below, the usual adrenalin effects were shown, but at room temperature even with low pressures of perfusion no constriction but only dilatation could be obtained. This absence of constriction with adrenalin at low temperatures is probably due to the pulmonary arterioles (which are peculiarly sensitive to variation of temperature) being already strongly constricted from cold and therefore unable to undergo further constriction. In the case of the blood perfused organ, as far as could be shown, the same failure to constrict occurred with fall of temperature, but the matter was here usually complicated by the necessity of using a high pressure of perfusion to force blood through the constricted vessels sufficiently rapidly for the purposes of the record. The absence of constriction is therefore in such a case due largely to the excessive pressure, and it appears probable that with temperatures above 20° C. the pressure is the chief factor in determining whether constriction is produced by adrenalin or not.

Fig. 10.

I would suggest therefore that this question of pressure might be the cause of the failure of Brodie and Dixon to obtain a constriction by this same method. The exact pressure at which they performed their perfusions is not definitely stated for lungs, but since they did not keep the temperature of the lungs constant by immersion of the animal in a warm bath, and since they actually stated also that the temperature of the perfusing blood might safely be allowed to fall several degrees, it seems likely that the temperature of the lung tissue was considerably below 37° C. A fall in temperature either of the perfusing fluid or of the lung itself produces vaso-constriction, and under these conditions therefore—as before described—in order to obtain a good flow through the lung vessels, a pressure certainly nearer 30 mm. than 15 mm. must have been employed. At such a pressure a constriction is only produced with very large amounts of adrenalin or suprarenal extract, and very rarely obtained with commercial adrenalin hydrochloride even undiluted (0·1 %). In this way a comparatively slight fall of temperature, insufficient in itself to cause a reversal of adrenalin action, may, by association with excessive pressure, prevent small constrictor actions from appearing.

Thus it seems clear that all preparations of adrenalin will, under conditions of pressure and temperature approximating to normal, cause constriction in the pulmonary circulation, though to a less extent than in most other organs.

To gain some idea as to what was the actual extent of the constriction and dilatation demonstrated by the records, the apparatus was calibrated, so that the flow at any instant during an experiment could be found by analysis of the curves. The following figures show the extent of the changes produced in the cat by the injection of varying amounts of adrenalin.

Exp. 1. Pressure = 10 mm. Hg.
Injection of 2 mgms. adrenalin hydrochloride (freshly made).

Before injection	flow = 26 c.c. per min.
10 secs. after injection	,, = 18 ,, ,,
15 ,, ,, ,,	,, = 9 ,, ,,
4½ mins. ,, ,,	,, = 16 ,, ,,
9 ,, ,, ,,	,, = 16 ,, ,,

Exp. 2. Pressure = 11 mm. Hg.
Injection of 0·4 mgm. adrenalin.

Before injection	flow = 34·6 c.c. per min.
15 secs. after injection	,, = 30 ,, ,,
25 ,, ,, ,,	,, = 10 ,, ,,
1 min. 35 secs. after injection	,, = 17 ,, ,,
2½ mins. after injection	,, = 22·6 ,, ,,
4 ,, ,, ,,	,, = 34·6 ,, ,,
6 ,, ,, ,,	,, = 40 ,, ,,
12 ,, ,, ,,	,, = 40 ,, ,,

Exp. 3. Pressure = 9 mm. Hg.
Injection of 0·2 mgm. adrenalin.

Before injection	flow = 30 c.c. per min.
25 secs. after injection	,, = 24 ,, ,,
35 ,, ,, ,,	,, = 11 ,, ,,
2½ mins. ,, ,,	,, = 26 ,, ,,
2 mins. 50 secs. after injection	,, = 30 ,, ,,
3½ mins. after injection	,, = 40 ,, ,,

Exp. 4. Injection of ·08 mgm. adrenalin.

Before injection	flow = 32 c.c. per min.
25 secs. after injection	,, = 29 ,, ,,
30 ,, ,, ,,	,, = 22 ,, ,,
1 min. 20 secs. after injection	,, = 29 ,, ,,
1 min. 25 secs. ,, ,,	,, = 32 ,, ,,
1 min. 35 secs. ,, ,,	,, = 35 ,, ,,
2 mins. after injection	,, = 55 ,, ,,
3 ,, ,, ,,	,, = 65 ,, ,,
5 ,, ,, ,,	,, = 65 ,, ,,

Reviewing these figures, it is seen that with injections of adrenalin ranging from 2 to 0·2 mgms. a constriction is produced which in every case cuts the flow down to about one-third of its original amount, the chief difference between the effects of stronger and weaker doses being in the length of time during which the constriction persists, and the amount of the after-dilatation. With less than 0·1 mgm. of adrenalin, the constriction is weaker, as well as of shorter duration, but the succeeding dilatation is more marked, increasing the flow to twice its original amount.

A criticism of this method which might be made is that since the pulmonary artery and its branches are all included in the preparation, the effects observed may be due to changes in the calibre of the larger pulmonary vessels and not of the arterioles. It was shown by Cow[1] in 1911, taking rings of the pulmonary artery or its branches, that adrenalin caused a constriction of these larger arteries, which appeared to get less as the ring of artery was taken nearer and nearer to the lung tissue. It was therefore suggested that in the still smaller arteries and arterioles adrenalin would have no effect at all.

Two methods were adopted in the present research to test the possibility that the effects noted were due to the action on the pulmonary artery and not on arterioles. In the first place it was shown that precisely similar effects with adrenalin were obtained whether the cannula was inserted in the main artery or in one of its branches as close as possible to the lung tissue, that is, whether the whole length of the pulmonary vessels was exposed to the adrenalin or not.

A second test was made by perfusing the drug through the large vessels only, and not through the arterioles and capillaries. This was done by inserting the cannula in the pulmonary artery in the usual way and ligaturing off all except one of the small branches of the artery just before their entrance into the lung tissue. The single branch left unligatured was dissected out as far as possible towards its division into still smaller vessels and a cannula tied into it, pointing towards the heart. This cannula was connected to a glass tube, drawn out to a very fine capillary end and packed fairly tightly with glass wool (thus introducing an artificial peripheral resistance) and this in its turn connected to the tube leading into the receiver (*A*). Perfusion was performed as before, but in this case the blood passes only through the pulmonary artery and its larger branches and not through the arterioles. The resistance is adjusted (by varying the tightness of packing of the glass wool) until the outflow of blood approximates to that usually obtained through the lung tissue with the same pressure. Injection of adrenalin, even up to 2 mgms., produces hardly any effect on the flow (Fig. 11), thus showing that the usual results must be due to an action on the arterioles and not merely on the large arteries.

---

[1] Cow. This *Journal*, XLII. p. 125. 1911.

Fig. 11.

The next point to be investigated was the question as to whether the action of adrenalin could be taken as giving conclusive evidence of the existence of vaso-motor nerves. Electrical stimulation of nerves was tried by Brodie and Dixon with negative results; but it seemed possible that the lack of effect might be due to the same causes as in the case of adrenalin, and that stimulation at a higher temperature and a lower pressure might be effective. A means had therefore to be found of stimulating the nerves while the animal was immersed in the warm saline bath. For this purpose the Keith Lucas electrodes were adopted, since they can be used for stimulation under water. These were placed in position on the nerve under investigation several minutes before stimulation was commenced, in order to allow the flow again to become constant after the disturbance of temperature caused by the adjustment of the electrodes. Stimulation of the following nerves by rapidly repeated induced shocks was tried: sympathetic chain at various levels, stellate ganglion and its branches, inferior cervical ganglion and its branches, cervical sympathetic and the vagus nerve. In the case of the vagus, sympathetic chain, inferior cervical ganglion and cervical sympathetic no effect on the pulmonary flow was produced, but on stimulation of the stellate ganglion itself, or of its branches running towards the heart and lungs, or of the sympathetic chain immediately below it, a slight constriction was in some cases obtained (Fig. 12). In two experiments, stimulation of the stellate ganglion produced a weak constriction followed by a small dilatation, suggesting a feeble imitation of an adrenalin curve, and in several other cases quite a marked dilatation was repeatedly obtained (Fig. 13). The effects were however too slight and too uncertain to be altogether satisfactory, or to allow of very definite conclusions being drawn. It appeared possible that the difficulty of producing constriction or dilatation might be due to the opposing action of constrictor and dilator nerves, since the effect of adrenalin points to both constrictors and dilators being sympathetic in origin. Attempts were therefore made, by varying the strength of the stimulus and the rate of stimulation, to stimulate the one set of nerves without the other, but the effects were still just as slight and as uncertain.

Fig. 12.

Another possibility was that the presence of serum in the defibrinated blood used for the perfusion might have a deleterious effect upon nervous structures, and thus prevent the action of the nerves. A few experiments were therefore performed with hirudinised instead of defibrinated blood, but again no very certain effect could be shown, only the same very slight constriction on stimulation of the stellate ganglion. Hence the results of stimulation of nerves do not give indubitable evidence of the existence of vaso-motor nerves to the lungs, but it may be that the nerves do not survive a sufficient length of time under the conditions of a perfusion experiment to respond to electrical stimulation.

Another way of investigating the question as to whether the action of adrenalin indicates the existence of nerves or not was suggested to me by Dr F. Ransom. This was by the use of ergotoxin. Since this drug paralyses motor but not inhibitor nerves belonging to the sympathetic system, it seemed that if the adrenalin effects are due to nervous action, after ergotoxin the constrictor effect should be lost. For this purpose the mode of procedure was as follows:

Fig. 13.

The animal (cat) was pithed, artificial respiration performed and a cannula inserted in the external jugular vein. From 10 to 15 mgms. of ergotoxin phosphate[1] (about 5 mgms. per kilo. of the animal's weight), dissolved in distilled water with the addition of a drop of 10 °/₀ NaOH, were then slowly injected intravenously, and an interval of 10 minutes allowed to elapse to give time for the circulation and action of the drug. At the end of that time the animal was bled and a perfusion experiment performed in the ordinary way. It was found that after such an injection of ergotoxin 0·2 mgm. of adrenalin gave no constriction at all, but an immediate and very marked dilatation (Fig. 14) much more sudden in its onset and more violent and prolonged than the dilatation which usually follows the adrenalin constriction.

---

[1] The ergotoxin phosphate was kindly given to me by Dr H. H. Dale, from the Wellcome Research Laboratories.

This affords very strong evidence in support of the view that the usual constriction obtained with adrenalin is due to stimulation of vaso-constrictor nerve endings, and that the pulmonary arterioles, like those of the majority of other organs, are supplied with constrictor and dilator fibres, both of which in this case belong to the sympathetic system and are therefore stimulated by adrenalin. The effect produced by adrenalin is thus the resultant of the antagonistic action of two sets of fibres, constriction or dilatation respectively being obtained, according to the amount of adrenalin injected and the previous condition of the arterioles.

Fig. 14.

CONCLUSIONS.

1. All preparations of adrenalin, in amounts not less than 0·01 mgm., under normal conditions of pressure and temperature, produce constriction of the pulmonary vessels of dogs, cats, rabbits, rats, guinea-pigs and ferrets. This constriction is followed by a dilatation, especially marked in cats.

2. The minimum amount of adrenalin capable of producing constriction is 0·01 mgm., but dilatation is obtained with only 0·00002 mgm.

3. The effects of adrenalin are due to action on the smaller arteries and arterioles, not merely on the pulmonary artery itself.

4. Stimulation of the stellate ganglion produces slight vaso-motor effects.

5. After ergotoxin, the constrictor effect of adrenalin is lost, only dilatation being produced.

6. It is concluded that the constriction is due to vaso-constrictor nerves of sympathetic origin.

I wish to take this opportunity of thanking Dr Winifred Cullis for help and advice during this research.

(Part of the expenses of this research was defrayed by a grant from the Government Grant Committee of the Royal Society.)

ON THE EFFECTS OF COMPLETE AND INCOMPLETE CASTRATION UPON HORN GROWTH IN HERDWICK SHEEP. By F. H. A. MARSHALL AND J. HAMMOND.

As has been pointed out in a previous paper[1], in Herdwick sheep there is a well-marked sexual differentiation, since the rams are horned and the ewes hornless. It was shown further that castration inhibits horn growth forthwith and at any stage of development. In the present paper these results are confirmed and extended.

Effect of complete castration.

The two Herdwick rams (born in May 1910 and May 1911 respectively) and the three Herdwick wethers referred to in the previous paper are still alive. The horns of both the rams are now very well developed, but those of the wethers have undergone no appreciable further growth. The first wether (born in May 1910) still has low, rounded knobs, while the horns of the other two (born in May 1911) are about 4¼ and 6 inches long respectively, these being their lengths at the time of castration.

Two more ram lambs, born in May 1912, were castrated in the following September, the testes and epididymes being completely removed. On the day of the operation their horns were measured, and found to be as follows:—

(1) Right horn, 5 inches; left horn, 5 inches.
(2) Right horn, 4¾ inches; left horn, 4 inches.

Their lengths at present (March 1914) are as follows:—

(1) Right horn, 5⅜ inches; left horn, 5½ inches.
(2) Right horn, 4½ inches; left horn, 4¼ inches.

It is thus seen that the lengths of the horns in the two wethers have not appreciably altered, and the results fully confirm those recorded in the previous paper. The horn lengths should be compared with those of the four animals of the same age described below.

---

[1] For references, see end of this Paper.

Effect of unilateral castration.

In support of the view that the relation between the gonads and the secondary characters of sex is nervous rather than through the influence of internal secretions it has been alleged that in certain cases of hermaphroditism or of unilateral castration there is a direct correspondence between the sex of the gonad and the secondary characters on the same side of the body as the gonad. Thus Weber has recorded a case of a chaffinch which had a testis on the right side associated with right-sided male plumage and an ovary on the left side associated with left-sided female plumage. This case is quoted by Nussbaum, who regards it as evidence of the influence of the nerves upon the secondary characters of sex. Poll has described three cases of hermaphroditism in which the external characters on each side corresponded with the sex of the gonad on that side. Thus in a bullfinch the gonad and the plumage of the breast on the right side are stated to have been male while those on the left were female. In the case of a pheasant, recently described by Bond, the secondary sexual characters were unilaterally distributed, while there was a single gonad in which the male and female elements were gathered together and lying on the left side but nearer to the middle line than the normal position of the left sex gland.

Fig. 1.

Fig. 2.

Fig. 1. Herdwick Ram, normal, born May 1911.
Fig. 2. Herdwick Wether, without horns visible, born May 1910; castrated August 1910.

Among mammals Rörig has described cases of unilaterally castrated stags with abnormal antler development on one side, usually on the opposite side to that on which the testis was absent. Fowler has also stated that in fallow deer one-sided castration may result in the abnormal or incomplete development of one antler, the other antler being normal.

In our experiments one testis (together with the corresponding epididymis) was removed from each of two Herdwick ram lambs. The lambs were born in May and operated upon in the following September. The following are the records of the experiments:—

(1) September 1913. Right testis removed. Lengths of horns on day of operation, right, 5¼ inches, left, 6 inches. Lengths of horns in March 1914, right, 10½ inches, left 10⅞ inches.

Fig. 3.

Fig. 4.

Fig. 3. Herdwick Wether, born May 1911; castrated October 1911. The horns are the same length as they were at the time of castration.

Fig. 4. Herdwick Ram Lamb, born May 1913; one testis removed September 1913. The horns have continued to grow and are symmetrical.

Fig. 5.

Fig. 6.

Fig. 5. Herdwick Wether, born May 1913; castrated September 1913.

Fig. 6. Herdwick Wether, born May 1913. The testes were removed in September 1913 but the epididymes retained.

(2) September 1913. Left testis removed. Lengths of horns on day of operation, right, 4½ inches, left, 4⅛ inches. Lengths of horns in March 1914, right, 8¼ inches, left, 8½ inches.

It is thus seen that the removal of one testis only had no effect upon the symmetry of the horns. It is also seen that one testis only was able to supply sufficient stimulus to permit of the continuance of the horn growth. It is to be remarked, however, that the horn growth was much slower than that of the normal rams previously described. In one of these each of the horns was already eleven inches long in December when it was only seven months old. Possibly however there may be considerable variation in the rate of horn growth in normal Herdwick rams.

Effect of removing the testis but retaining the epididymis.

It has been suggested that the epididymis may exert a controlling influence on the development of the secondary male characters (Biedl), but so far as the authors are aware there is no actual evidence that this is the case. It seemed desirable, however, that the matter should be put to the test in Herdwick rams by removing the testis on each side but leaving the epididymis in position. This was accordingly done in the case of two male Herdwick lambs which were born in May and operated upon in September. The following are the records of the horn lengths on the day of the operation and at the present time:—

(1) September, right horn, 5½ inches, left horn, 6¼ inches. March, right horn, 6 inches, left horn, 6¼ inches.

(2) September, right horn, 2¾ inches, left horn, 2⅞ inches. March, right horn, 3⅛ inches, left horn, 3¼ inches.

It is thus seen that there was practically no further horn growth after castration, and thus, that the removal of the testes alone without the epididymes was sufficient to inhibit such growth. The epididymes in the two wethers can still easily be felt in the scrotal sacs.

Discussion.

Geoffrey Smith as an alternative to the hormone hypothesis regarding the relation between the gonads and the secondary sexual characters has put forward another theory which he calls the theory of metabolic stimulation. According to Smith's view "the gonad, by feeding on certain specific substances in the blood, stimulates the overproduction of these substances by the metabolic organs, and so brings about changes in the composition of the blood, which act as a stimulus on the secondary sexual characters." This theory in another paper is fully elaborated for certain Crustaceans, in terms of fat metabolism, but Smith is careful to state that he regards the changes in the fat content of the blood which this theory postulates (and for which there is actual evidence based on estimation experiments) as merely an accompaniment of more deep-seated changes. It is an essential part of Smith's theory that fat molecules together with "fat-links" are given off from the liver and carried to the gonad which absorbs the molecules but releases the links. The latter are supposed to pass back to the liver and to fix on to new fat molecules which are borne to the gonad as before. This continued process is believed to lead to the regeneration of new fat-chains in the liver, and the result of the whole series of changes is to flood the blood with a large number of fat-link and fat combinations and of free fat-links. These are supposed to stimulate the development of the secondary sexual characters. It is to be observed that since the giving off of free fat-links by the gonad is an essential part of the theory, the gonad on this view is an organ producing an internal secretion which is in some way necessary for the growth of the secondary characters.

The principle underlying Smith's theory is the production in excess of substances which stimulate the development of the secondary sexual characters. This principle is held to be essentially similar to that upon which Ehrlich's side-chain theory of immunity is based. But according to the immunity theory the antibodies with which the blood is flooded remain there for some considerable time after the removal of the exciting cause which was originally responsible for their production. It is clear, therefore, that, on Smith's view, the removal of the testes from Herdwick rams ought to have no result (or at any rate no immediate result) upon the continuance of horn growth, since the specific substances called forth by the gonads would remain in the blood for some considerable time after castration; that is to say, horn growth in Herdwick rams ought to continue after the extirpation of the testes; but this, as has been shown, is not the case.

The results described in this paper clearly support the view that the testes elaborate one or more hormones or internal secretions, which, either directly or indirectly, act as a stimulus to the tissues from which the horns develop. It has not so far been found practicable to attempt to imitate the normal action of this problematical hormone, by injecting fresh extract of ram's testis. It may, however, be pointed out that if this were done over a considerable period, and the result were negative, this could not be regarded as antagonistic to the hormone theory, since there is really no sound reason for supposing that an emulsion or extract of testicular gland would contain the specific substance or substances unaltered, and in a condition capable of setting on foot the complicated nexus of metabolic processes which results in the development of the secondary characters of sex.

SUMMARY OF RESULTS.

Removal of the testes from Herdwick ram lambs arrests further horn growth forthwith and at any stage of development. Unilateral castration does not stop horn growth, neither does it affect the symmetry of the horns, but there is some evidence that the further development of the horns is slower than in normal unoperated animals. Removal of the testes without the epididymes (these being retained) inhibits horn growth in precisely the same way as ordinary castration in which both organs are removed.

The investigation was carried out at the Field Laboratories, Cambridge. The operations were performed by F. H. A. Marshall. The expenses for the investigation have been defrayed by grants made by the Government Grant Committee of the Royal Society, and by the Board of Agriculture and Fisheries out of money placed at their disposal by the Development Commission.

REFERENCES.

Biedl. "Innere Sekretion," Wien. 1911.

Bond. Journ. of Genetics, III. p. 205. 1914.

Fowler. Proc. Zool. Soc. p. 485. 1894.

Marshall. Proc. Royal Soc. B. LXXXV. p. 27. 1912.

Nussbaum. Ergebn. d. Anat. u. Entwick. XV. p. 39. 1905.

Poll. Stzngsbr. Gesel. Natur. f. Freunde zu Berlin, p. 338. 1909.

Rörig. Arch. f. Entwick.-Mech. VIII. pp. 382, 633. 1899. Ibid. X. pp. 525, 618. 1900. Ibid. XI. pp. 65, 225. 1901.

Smith. Linnean Society. March, 1913. Quart. Journ. Micr. Science, LIX. p. 267. 1913.

Weber. Zool. Anz. XIII. p. 508. 1890.

THE CARBON DIOXIDE PRODUCTION OF HEAT RIGOR IN MUSCLE, AND THE THEORY OF INTRA-MOLECULAR OXYGEN.

By W. M. FLETCHER AND G. M. BROWN.

(From the Physiological Laboratory, Cambridge.)

CONTENTS.

		PAGE
I.	The CO_2 discharge of heat rigor	183
II.	The CO_2 discharge at temperatures between 40° and 100° C.	186
III.	The alleged 'fixing' of the CO_2 precursor by rapid heating	189
IV.	Experimental modifications of the CO_2 discharges of heated muscle	192

UNTIL quite recent years the views of muscle physiology commonly associated with the names of Hermann and of Pflüger, have been everywhere predominant. The discharge of energy by the muscle cell—and by inference its discharge by any living cell—has been supposed to depend upon the dissociative breakdown of some labile molecule (Hermann's 'Inogen') in which oxygen had taken its place beforehand (Pflüger's 'intra-molecular' oxygen) in such a manner that upon the dissociation of the molecule the energy yielded was energy due to a combustion, and the final products, carbonic acid and water, represented the results of a combustion. The product lactic acid, which was supposed to arise from the same labile molecular complex, has been variously regarded either as another final product, or as an intermediate product destined possibly to be used in future reconstruction of the Inogen complex, or again as marking only a stage of incomplete combustion due to insufficient oxygen supply.

This view of muscle chemistry has rested almost entirely upon two main groups of experimental evidence. Of these the first and chief is the group of facts which have appeared from the earliest times to show that the discharge of energy by the muscle, and the yield of the final product CO_2, are alike independent—within wide limits—of a contemporary and immediate oxygen supply. Spallanzani (1803) for lower animals, Liebig (1850) and Matteuci (1856) for the case of frog's muscle, showed this long before Hermann did, and by experimental methods at least as satisfactory as his. The simple fact that a stimulated muscle, or even a resting muscle, will continue to yield CO_2 after it has been deprived of oxygen, has been interpreted almost universally throughout the last century to mean that oxygen had been stored in the muscle in some way beforehand—that it was actually present already in some 'intra-molecular' form. Historically this interpretation has had overwhelming influence in shaping physiological views of the most elementary vital processes, and by no means only those of the muscle cell.

In the second group on the other hand have been the results of heating muscle. **Hermann**[1] believed that rapid heating 'fixed' the Inogen molecule from which lactic acid and CO_2 together came, and that a muscle rapidly heated yielded no CO_2 just as it yielded no lactic acid. He believed further that the CO_2 of heat rigor which, as he showed, was diminished by previous fatigue, was so diminished not because CO_2 as such had been driven off from the muscle during the fatigue, but because the store of Inogen from which it came in heat rigor had already been called upon and diminished by the previous contractions. **Pflüger**[2] also took the same two bases for his idea of intra-molecular oxygen, and he gave particular attention to the results of heating muscle. He interpreted the increase of the CO_2 yield on heating as due to an increased dissociation of its previously oxidised precursor, and he gave evidence, which will be examined further below, to show that the CO_2 yielded by warmed muscle was in reality newly dissociated CO_2 and not an expulsion of CO_2 already present as such in the muscle.

The first of these fundamental groups of experiment, those dealing with the behaviour of the muscle with and without oxygen, has already been found inadequate to support what has been for so long the standard theory of muscle physiology. Work done in recent years in this laboratory upon the relations of oxygen supply to the CO_2 discharge by muscle, to the lactic acid yield, and to the heat production of muscle, has pointed with accumulating effect to a widely different view, according to which lactic acid as such appears and disappears in the tissue as an incident of the machinery of the muscle, in which it or its hydrogen ion plays an essential part. The CO_2 on the other hand arises from a molecular system, not necessary identical with that concerned in lactic acid formation, where combustion by immediate and not antecedent

[1] **Hermann.** *Untersuchungen über den Stoffwechsel der Muskeln.* Berlin, 1867.

[2] **Pflüger.** *Pflüger's Archiv*, XVIII. p. 247. 1878.

oxidation supplies energy for the restoration of the lactic acid to its former position in its precursor, and for the restoration of the *status in quo ante* to the muscle as regards its chemical and physical properties. Upon this view the CO_2 is not yielded as the result of dissociation of a molecule already containing 'intra-molecular' oxygen, but appears always as the result of a direct combustion due to a contemporary supply of oxygen, except when, in much smaller amounts, it may appear from a muscle previously saturated with it, whenever circumstances allow lactic acid so to accumulate in the muscle as to displace it from a chemical or physical combination. This expulsion of ready formed CO_2 from the muscle tissues by increase of acidity provides an obvious interpretation, curiously left out of account in the past, for the fact that a muscle withheld from oxygen may nevertheless continue to yield CO_2. We have just seen that it was this experimental fact, under the alternative interpretation in terms of intra-molecular oxygen, that has been the chief basis of the Inogen theory.

The other class of evidence however, namely that got by the study of heated muscle, still remains in the field to support the older view of Hermann and his followers, and the object of the present paper is to examine it.

In a former paper by one of us[1] some account was given of the CO_2 discharge from muscle at different temperatures. The rate of discharge during heat rigor at 40° C., was determined and the total output was estimated to be about 30 °/₀ by volume of the muscle substance. It was shown also that on rapid heating to 70° C. or 100° C., no 'fixation' of the substance yielding CO_2 appeared to happen, and a definite contradiction was given to the common statement first made by Hermann that a muscle plunged into boiling water gives off no CO_2 at all. The discharge from the rapidly heated muscle undergoing heat coagulation at 100° C. was greater than that due to heat rigor at 40° C., though the discharge was completed more quickly, and no reason was found then to suppose that it did not include the discharges which lower temperatures would have produced.

These experiments were only preliminary and were done moreover at a time when no quantitative knowledge was available of the relations of lactic acid production to temperature. They have now been repeated with the use of slightly different methods, and have been extended with special reference to the results obtained by Hermann and by Pflüger, and to the interpretations formerly placed upon these.

---

[1] Fletcher. This *Journal*, XXIII. p. 87. 1898.

Methods. In the simplest form of experiment the method was in all essentials the same as that previously used by one of us[1]. The muscle for estimation was placed in a simple tubular chamber through which an aspirator drew a current of pure air. The air as it passed towards the aspirator was drawn to bubble through standard baryta solution contained in two or more absorption chambers arranged in series. The loss of baryta on precipitation of carbonate due to CO_2 in the current, was determined by titration with standard hydrochloric acid, with phenolphthalein as indicator. As in most cases the chief object was to secure the absorption of the total CO_2 discharged under given conditions, and not to determine the discharge for short periods of time, it was found convenient to use stronger baryta solutions than those taken in the earlier experiments. In general the standard baryta solution was between N/4 and N/5, and with this concentration the aspirator current can be run much faster through the absorption chambers without escape of CO_2 due to incomplete absorption.

The absorption chamber was made so that titration could be effected in it subsequently, with minimal exposure to atmospheric CO_2. It is shown in Fig. 1. The entering current, bringing CO_2 from the muscle chamber, passes in bubbles through about 15 c.c. of standard baryta solution. The bell mouth of the delivery tube checks the formation of each bubble and gives added time for CO_2 absorption. Experience shows that the absorption is complete even at relatively fast rates of current. Where two or more chambers are used, as in the case of every experiment to be given, any traces which escape the first chamber in the series are absorbed in the second chamber. Even at very rapid rates of flow the third chamber in the series, always used as a control, has remained free from any traces of precipitate and has given a zero result on titration.

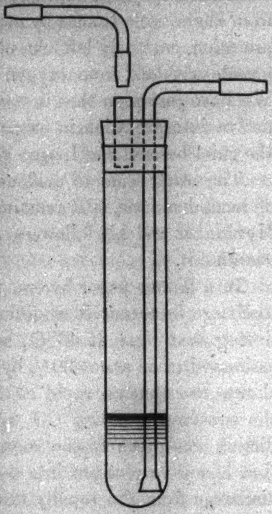


Fig. 1.

When estimation is desired, whether before or after the end of an experiment, a chamber is clipped off and removed, while another charged with fresh standard baryta is put in at any desired point of the series in place of it. For titration the exit tube is removed, but the clip is left upon the rubber attachment of the entry tube. Addition of indicator and of acid is now made through the tubular opening left in the rubber cork of the chamber. Movement of the contents of the chamber for stirring is necessary for proper titration, and this can be secured by tilting the chamber in the hand while the open tube is closed by the finger. Pinching the clipped rubber tube of the entry tube will bring what fluid may be held in the mouth of the entry opening into the general bulk of solution from time to time when the end point of titration is approached and serves also to bring back into the chamber any fluid film across the titration opening. If the neutral point has been passed on the acid side it can be brought back by addition from the baryta burette. With a little practice these chambers have been found to be very convenient for rapid and accurate titration. Control titrations for standardisation of the standard solutions have always given closely consistent results.

---

[1] *loc. cit.*

When the neutralised tube is rinsed out for filling again, the baryta is added not through the opening in the rubber cork, but directly to the opened chamber. The very slight loss due to atmospheric CO_2 during this charging of the chamber occurs equally when control titrations are to be made for standardisation of the solutions, so that it does not affect the results of estimations in experiment.

The aspirator used was a large form of Mariotte's bottle from which the flow of water could be regulated by alterations in the length of siphon, and its rate determined by volume measurement in a given time. For many experiments however it was found convenient to use a water-pump instead of the aspirator bottle. The pump was set to produce a large negative pressure which was put into connection with the series of absorption tubes through a screw tap. By adjustment of the tap the rate of air current through the absorption tubes could be regulated with accuracy and remained constant within narrow limits under all the normal variations of water pressure at the head of the pump.

A difficulty arises with regard to the determination of the actual weight of muscle used in an experiment. The muscle obviously cannot be weighed directly except by removal from the limb bones with serious injury and consequent chemical change. In the observations by one of us already referred to, the uninjured limbs were taken, and comparative absolute amounts of CO_2 were registered under different conditions.

A. V. Hill[1] in his observations upon the heat production by muscle, used uninjured limb muscles and expressed his results in calories per c.c. of the whole limb tissues. The weight of the whole hind limbs, or of whole frogs where these were used, he got by volumetric measurement of water displacement in a cylinder, on the assumption that the specific gravity of the tissues is nearly unity.

The products of muscular changes should be expressed of course in relation to the weight of muscle alone. This we did in many of our experiments by taking 'crossed' pairs of limbs, using one pair for the actual experiment while the other similar pair was stripped clean of muscle and the muscle weighed. We found by keeping the record of many such weighings that the ratio of net muscle weight to the weight of the whole limb (skinned) is remarkably constant. In Fig. 2 some of these results taken at random from frogs of widely varying sizes are set out graphically, and it will be seen that all lie close to the line representing the ratio 5 : 6. When we speak of 'limbs' we mean always the

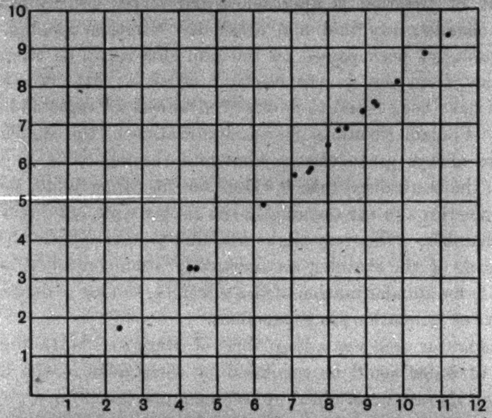


Fig. 2. The abscissæ represent weights in grammes of hind limbs prepared according to the description given in the text. The ordinates represent the weights in grammes of the whole muscle substance removed from the limbs so prepared. Each point shown gives the result of weighing the limbs and muscle substance respectively in a given case. The points fall close to the diagonal of the figure representing the ratio limbs : muscle = $\frac{6}{5}$.

---

[1] A. V. Hill (This *Journal*, XLIV. p. 466. 1912) and R. A. Peters (*Ibid.* XLVII. p. 243. 1913), in their respective papers upon heat production, have used limbs shortened not at the tibio-tarsal, but at the tarso-metatarsal joint.

pair of hind limbs removed by a cut across the pelvis close above the limbs, and deprived of their extremities by a cut across the tibio-tarsal joint[1]. In our experience it has been found best, and certainly most convenient, to weigh the limbs, so prepared, and to assign $\frac{3}{5}$ths of the weight to muscle substance. Fig. 2 shows how close an approximation this gives to reality, and where comparative estimations are to be made, the use of this muscle-limb ratio is even to be preferred to direct weighing. It avoids the irregularities which weighing the separated muscles may introduce through variations of dissection and of drying on different occasions, at different temperatures, and perhaps in different hands. In single pairs of comparative estimations we have used 'crossed' pairs of limbs to give exact equality of sample.

Other details of method will be given in connection with definite experiments in their place.

I. The CO_2 discharge of heat rigor.

We have made determinations of the yield of CO_2 from the frogs' limb muscles given during the progressive stages of spasm or contracture and later of stiffness and opacity which are seen in the muscle to follow in rapid succession as the temperature rises from above 30° C. towards 40° C. At 40° C. the establishment of full heat rigor is almost instantaneous, as is well known. We may say at once that we find that the discharge of CO_2 (like the yield of lactic acid) is affected only in its rate but not in its total amount by differences in the rapidity of infliction of temperatures between 30° and 45° C. Whether a muscle is given immediate heat rigor at 40–45° C. or is allowed to approach it more slowly at 35° C. the total products are the same.

In one series of observations the limbs immediately after excision were placed in a thin walled tubular chamber, the air current was drawn through it and through the absorption chambers, and the tubular muscle chamber was then immersed in the water bath kept at 40°.

In another series the limbs were put in a flask so as to lie submerged in ·73 °/$_o$ NaCl solution previously freed from CO_2. The air current was drawn so as to bubble through the salt solution into which the CO_2 as it is delivered by the muscles becomes diffused. This method is the same as that used in the experiments to be described later. It gives the same total results as the former method but the initial rates of appearance of the CO_2 in the absorption chamber are not so high.

By both methods an immediate high rate of CO_2 discharge from the muscle entering heat rigor is observed, but this rate rapidly declines, as one of us has previously shown, by exponential decrements, until the last traces of free CO_2 have diffused from the muscle substance to the air current. The amounts collected in successive periods with a rate of current of about 200 c.c. per hour are as follows in a typical case:

---

[1] See footnote on p. 182.

1st period,	1½ hours,	2·125 c.c. CO_2	= ·71 cc. CO_2	per ½ hour.
2nd ,,	1½ ,,	·51 ,,	= ·17 ,,	,,
3rd ,,	1 ,,	·21 ,,	= ·10 ,,	,,
4th ,,	1 ,,	·10 ,,	= ·05 ,,	,,

The net muscle weight was 8·16 gms.

It is not practicable to prolong the collection indefinitely until the final traces of CO_2 have escaped by diffusion, because bacterial changes and the large CO_2 production associated with them soon begin of course at this temperature. To determine the total CO_2 due to heat rigor we have used a rate of air current as rapid (250–350 c.c. per hour) as is compatible with complete absorption and have collected the total CO_2 for 4½ hours. A control absorption has then been made for half an hour and if the rate of yield has been found to be less than ·05 c.c. per half hour we have felt free to assume that our collected total (1) represents the whole discharge of heat rigor, diminished only by small traces left uncollected amounting to 1–5 % of the whole, and (2) does not include the results of bacterial change. Such determinations give for autumn frogs the following results:

Limb weight	Calculated muscle weight	Total CO_2 yield	CO_2 yield in c.c. per hundred gms. of muscle
9·6 gms.	8·0 gms.	2·94 c.c.	36·7 %
10·1	8·41	3·04	36·2
10·1	8·41	2·85	33·9
8·6	7·18	2·73	38·0
8·3	6·92	2·73	39·4

It will be seen that these percentage totals are all closely of the same order, none departing widely from the average.

We have seen that the total collected CO_2 is an approximation only, though a close one, to the total discharge. The weight of the limbs taken for the expression of a percentage result is again an approximation to reality, and the actual weighing on which it is based is necessarily open to fluctuations from reality due to differences of moisture. When it is important to secure strictly comparative results, as in some of the experiments to be given below, the greater part of the errors suggested can be avoided by using in a pair of experiments 'crossed' pairs of limbs, and by securing identity of conditions and treatment, and equality of periods of absorption, throughout the experiments.

For constancy of results it is important that the excised muscle should be enclosed within the experiment chambers as soon as possible, or at least at a fixed routine time after excision. If time be allowed for the survival yield of CO_2, which will vary according to the room temperature, to escape to the air, the total CO_2 for heat rigor subsequently will be proportionately diminished. Thus a delay of half an hour between excision and heat rigor at a room temperature of (say) 18° C. will diminish the total CO_2 yield for heat rigor by about ·25 c.c. On the other hand the constancy of the total yield, as mentioned already, is not affected by the rate at which the temperature is raised to 40° C. to produce heat rigor.

The results got by **Hermann**[1] do not at all correspond with those given here. Enough details of his method are not given to allow an estimate of the extent to which CO_2 escaped from the muscle in the processes of mincing and grinding in the frozen state, either to the air or to the freezing mixture used, before his collection of gas by the air pump began. He gives no series of similar observations and no constancy is noticeable in the results of different experiments. His total collected from 41·4 gms. muscle heated to 50° C. was 1·588 c.c. CO_2, or 4·1 % of muscle weight, while the total he gives for another similar experiment, at 45° was 8·9 %.

The average yield of CO_2 in heat rigor is seen from the table of our results to lie between 35 and 40 c.c. % of the muscle weight, and this may now be compared with the estimates previously made of other events in the muscle. According to **Fletcher** and **Hopkins**[2] the lactic acid yield of similar muscle reaches a very constant maximum of ·4 % lactic acid for autumn and of about ·3 % for spring frogs.

All the evidence we have from a study of the production of CO_2, of lactic acid, and of heat by the muscle indicates[3] that the events of heat rigor at 40° C. are different not in kind but only in velocity from those going on (if we exclude the presence of oxygen) in excised muscle at lower temperatures. The slow production of lactic acid from its precursor at room temperature is hastened as the temperature rises, and its formation appears to be almost instantaneous at 40° C. It has been pointed out already that the chief part of the survival CO_2

---

[1] *loc. cit.* [2] **Fletcher and Hopkins.** This *Journal*, xxxv. p. 267. 1907.

[3] See **Fletcher and Hopkins.** *Op. cit.* p. 275. **A. V. Hill.** This *Journal*, xliv. p. 466. 1912. **Fletcher.** *Ibid.* xlvii. p. 374. 1913.

output of excised muscle, whether amphibian or mammalian—indeed the whole of it if oxidation and bacterial processes are excluded in the early and late stages of survival history respectively—is due to displacement of preformed CO_2 by the increasing acidity due to liberation of lactic acid. There is no necessary reason to suppose that *pari passu* with the yield of lactic acid in the breakdown of the lactic acid precursor, a fresh yield of CO_2 is also taking place. In the same way, during heat rigor, when the chemical events seem to be the same as those of survival at lower temperatures except in velocity, we find no reason—and certainly no necessity—for assuming any fresh formation of CO_2. In the autumn frog muscle ·4 °/o lactic acid is produced in heat rigor and at the same time about 35 °/o CO_2. But this amount of CO_2 will be liberated from combination with sodium as bicarbonate by ·143 °/o of lactic acid. In the presence of phosphates, and perhaps also on account of the presence of proteins, we cannot suppose that the whole of the yield of lactic acid is available for the expulsion of CO_2 but there is at least enough to account for the relatively small output of CO_2 actually found.

The results of adding to the muscle acid from outside are given in a later section.

II. THE CO_2 DISCHARGE AT TEMPERATURES BETWEEN 40° AND 100° C.

When heat rigor is complete, and all but the last traces of discharged CO_2 have been collected, a further yield of CO_2 may be obtained by raising the temperature of the muscle through the temperatures at which the muscle proteins are coagulated. No further production of lactic acid occurs on the other hand, it will be remembered, as the muscle is heated from 40° to 100° C.

The collection of the CO_2 yielded at 100° does not raise the difficulties found at 40°, for as there is no possibility of bacterial change at the higher temperature, that absorption can be continued until the last traces of CO_2 have been yielded. Under the conditions described only minute quantities reach the absorption tubes after the third hour and after the fourth hour rarely any at all.

The following table gives the result of this further heating of the muscle.

Limb weight	Calculated muscle weight	Yield of CO_2 for heat rigor (40°)	Additional yield on boiling	Total CO_2 collected
9·6 gms.	8·0 gms.	36·7 °/o	28·5 °/o	65·2 °/o
10·1	8·41	36·2	39·9	76·1
10·1	8·41	33·9	36·6	70·5
8·0	6·65	not separately collected	not separately collected	70·9
6·3	5·25	,,	,,	73·8

These figures show the existence of a source, or sources, from which CO_2 may be discharged at higher temperatures quite independently of that yielding CO_2 in heat rigor at 40° C. This independent and additional discharge of CO_2 on heating from 40° to 100° C. is seen to be in general at least as great as that accompanying heat rigor.

To Hermann and his followers the supposed explosive dissociation of CO_2 which took place at 40° C. exhausted the possibilities of CO_2 production by the muscle substance. This supposed maximum yield at 40° has been indeed compared in the past with a fermentative yield having its optimum temperature near 40° C., its minimum near 0° C. and its maximum temperature below 70° C.

Obviously we have to consider the nature of the discharge, or discharges, between 40° and 100° as different altogether from that at 40°. Evidence converging from several independent directions will be given below confirming the suggestions already made that the discharge at 40° is not due to an explosive dissociation but rather to an expulsive separation from the muscle of CO_2 already held there as such, by the acid which then arises. But it is known that no further development of acid takes place as the muscle is further heated to 100° C., and if time be given, as it was given in the experiments here described, for the completion of the expulsion process at 40°, then no reason is left for assigning the later discharge at the higher temperatures to further expulsion by any increase of acid.

It has been seen already that a small part only—about a quarter—of the acid produced in heat rigor at 40° would be enough by itself to account for the whole heat rigor discharge of CO_2, on the supposition that the CO_2 is expelled from such a combination as that with sodium as bicarbonate; and it will be shown later that addition of more free acid gives at once a further yield of CO_2. There is then left in the heat-clotted muscle at 40°, in spite of its attained lactic acid maximum, a considerable quantity of CO_2 held as such in the muscle substance. No increase of acid occurs on further heating towards 100°, but other important changes do take place: among these the most obvious for consideration here are the physical changes of the heat coagulation of the muscle proteins, which are completed certainly at 75° C., and secondly the chemical changes which at 100° may be expected to affect the amino-acid combinations with CO_2.

It is probable that the physical changes of coagulation at temperatures between 55° and 75° C. include a complete alteration of the capacity of the protein constituents of the muscle for holding

CO_2 and it can be shown that if the muscle be heated to 75° C. and kept heated until the discharge at that temperature be finally completed, a fresh additional discharge occurs on the temperature being raised to 100°. Examples of this may be given[1].

Calculated muscle weights	CO_2 discharge at 75° C. collected till final completion	CO_2 discharge at 100° C.
7·80 gms.	(8 hrs.) 4·72 c.c. = 61·8 %	—
7·42	(not separately collected)	(8 hrs.) 6·08 c.c. = 81·2 %
5·14	(6½ hrs.) 3·55 c.c. = 63·3 %	—
4·30	(not separately collected)	(6½ hrs.) 4·00 c.c. = 85·2 %
7·14	(7¼ hrs.) 4·10 c.c. = 57·5 %	(4 hrs.) 2·20 c.c. = 30·9 %
6·86	(7½ hrs.) 3·90 c.c. = 56·9 %	(4 hrs.) 1·09 c.c. = 30·9 %

It may be suggested that the discharge of CO_2 at 100° is to be assigned to cleavage of carbamino groups in the protein constituents of the kind described by Siegfried[2], who showed that in the case of many amino-acids, as well as of polypeptides and of native proteins, CO_2 may be fixed by formation of carbamino-acids and subsequently liberated on heating or the addition of acid. He pointed out that a temporary accumulation of CO_2 during the oxidation processes of muscle might be organically combined in this way, and liberated subsequently by hydrolytic cleavage independently of any further oxygen supply.

It is not our present object however to analyse in detail the origins of the discharge of CO_2 at the higher temperatures. Certainly that analysis should not begin with the study of the CO_2 dissociations from a very complex substance like that of muscle, until more elementary knowledge of the relations, physical and chemical, of simple proteins in respect of combination with CO_2 have been ascertained under easily controlled conditions.

For our present purpose we are only concerned to show that there is a discharge of CO_2 at the higher temperatures which is fairly constant in percentage amount for similar muscles and which is different in origin—as will appear further below—from the discharge of CO_2 characteristic of heat rigor at 40° C. Complete heat rigor does not, as Hermann, Pflüger and others have held, exhaust the capacity of the muscle for yielding CO_2 on heating.

---

[1] For these experiments spring frogs were used and it was noticed here, as in some other instances, that the discharges of CO_2 were on the whole higher than those for autumn frogs; this however is immaterial to the results of the paired observations made.

[2] Siegfried. *Ztsch. f. physiol. Chem.* XLIV. p. 85. 1905.

III. THE ALLEGED 'FIXING' OF THE CO_2 PRECURSOR BY RAPID HEATING.

In the last section it has been shown that the muscle slowly heated to 100° C.—slowly enough, that is to say, to allow the development of the heat rigor discharge as well as the discharge at the higher temperature—gives off a total of about 65–80 c.c. CO_2 per 100 gms. of muscle. We are now in a position to compare this maximum discharge with that obtained from muscle when 'scalded,' or heated as rapidly as possible to 100° C.

The common belief that a muscle rapidly scalded yields no CO_2 rests of course upon **Hermann**'s well-known studies in muscle metabolism[1]. His familiar hypothesis that lactic acid and CO_2 arise in contraction and in rigor from 'one and the same' cell constituent—the so-called 'Inogen'—of the muscle, made it almost inevitable that he should believe in the heat fixation of the CO_2 precursor, for **du Bois Reymond**[2] had shown earlier that a quickly scalded muscle was neutral to litmus paper though one allowed to pass into heat rigor at 40° C. was definitely acid. If the lactic acid precursor is 'fixed' on rapid coagulation, so must be the CO_2 precursor if that is the same substance, as **Hermann** held it to be.

It is not perhaps generally realised that though **Hermann** announced positively that a rapidly heated muscle yielded no CO_2, he gave no experimental demonstration whatever to show it. A muscle scalded by immersion in a large quantity of water freely boiling over a flame was said, on inspection, and without any process of estimation, to have 'lost no noteworthy quantity of gas.' The same muscle brought subsequently to the air-pump yielded no more CO_2 until acid was added, when a slight additional discharge occurred. In another series of experiments, muscle which had previously been chopped up, frozen and thawed, was raised, not rapidly, to 70° C. when it was found to give no further yield of gas to the receiver. In such a case, rigor no doubt had already become complete. In the only experiment of this kind of which actual estimations are quoted it is stated that 'longer warming at 45° C.' preceded the evacuation at 70° C. The only point made here appears to be that no fresh discharge of CO_2 occurred at 70° C.: the volume collected was that due to the heat rigor at 40° C. Though **Hermann** speaks of 'rapid heating to 60–70°' giving the same result, namely, fixation of the CO_2

---

[1] **Hermann.** *Untersuchungen über den Stoffwechsel der Muskeln.* Berlin, 1867.

[2] **du Bois Reymond.** *Berliner Monatsbr.* p. 307. 1859.

precursor, as rapid scalding, he mentions no case in which rapid heating to 70° was attempted.

So far as we are aware no evidence has been subsequently added to support Hermann's assertion, and it is remarkable that it has nevertheless continued to be almost a commonplace of physiological literature that a muscle rapidly heated yields no CO_2. On the other hand complete confirmation[1] has been found for the heat fixation of the lactic acid precursor first shown by du Bois Reymond.

In the experiments by one of us already quoted[2], the muscle was enclosed in a thin-walled experiment chamber which was immersed in boiling water when the effects of quick heating were to be observed. Against the results then obtained—which did not show the supposed 'fixing' effect of scalding—it might be urged that the muscle within the chamber was not heated rapidly enough to secure heat fixation of the hypothetical CO_2 precursor. We have therefore thought it desirable to repeat the observations by use of a method in which the muscle itself is brought directly into boiling salt solution. The experiments were made in duplicate; 'crossed' pairs of limbs giving closely similar preparations were used, of which one pair was brought at once into boiling water, while the other for control was allowed to pass through the stages of heat rigor and of protein coagulation successively by more gradual heating from room temperature to final boiling.

The muscle chambers were small (220 c.c.) Erlenmeyer flasks, containing 50 c.c. of NaCl solution (·73 %) previously freed from all traces of CO_2 by boiling while a current of pure air was drawn so as to bubble through it. One 'crossed' pair of limbs was added to the solution in one flask, the aspirator current started, and the flask raised by immersion in a water bath to 40–45° C. The entry tube to the flask reached its bottom, so that a stream of bubbles was drawn through the solution in which the muscles lay. It has been seen already that by this method the CO_2 leaving the muscle by diffusion can be collected as efficiently—if not as rapidly—as when the air current passes directly over the exposed muscle. When it was desired to record the CO_2 discharge due to heat rigor as such, the muscle chamber was maintained at 40–45° C. as described already, for 4–4½ hours and the resulting CO_2 estimated. After this the temperature was raised to boiling and so maintained until all the CO_2 for this further discharge had been collected in the absorption tube and estimated. When only the total of the discharges of heat rigor and of the events occurring in the passage from 40° to 100° C. was required, the flask was raised to boiling after long enough time—generally half an hour—had been given at 40–45° C. to secure that the processes of heat rigor had been completed.

---

[1] Fletcher and Hopkins. *Op. cit.*

[2] Fletcher. This *Journal*, XXIII. p. 87. 1898.

The other companion 'crossed' pair of limbs, on the other hand, was brought as quickly as possible to 100°. The other flask, made ready with exactly similar connections, was brought to boiling. The flask was removed momentarily from the flame, the limbs were dropped in so as to fall directly into the boiling water with a minimal delay in the steam, and the flask was instantly closed. At the same moment the aspirator current was started. The flask was kept just boiling for a few minutes over the flame and then placed in a bath maintained at 100° C. during the collection of the CO_2. We were satisfied that the manœuvre of 'scalding' the muscle in the manner described allowed no significant loss of CO_2 to the outside air. Any such loss, if it occurred, would diminish the total CO_2 collected and would favour not our view but the view that heat-fixation of a CO_2 precursor does exist. The exit tubes of both flasks were fitted with condensing water-jackets for the return of steam to the flasks. The results of the estimations were as follows:

Limb weights	Calculated muscle weight	Heat rigor at 40° C., then boiled	Rapid 'scalding' at 100°, then boiled
* 8·9 gms.	7·42 gms.	5·34 c.c. = 72·0 %	—
8·9	7·42	—	4·46 c.c. = 60·1 %
* 10·0	8·33	5·79 c.c. = 69·5 %	—
10·0	8·33	—	4·99 c.c. = 59·9 %
6·5	5·42	—	4·23 c.c. = 78·0 %
8·3	6·92	6·02 c.c. = 87·0 %	—
7·5	6·25	—	4·09 c.c. = 65·5 %
* 8·7	7·27	5·6 c.c. = 77·1 %	—
8·7	7·27	—	4·70 c.c. = 64·6 %
5·10	4·25	—	3·10 c.c. = 72·7 %

* Crossed pairs of limbs.

The figures given have shown how completely at fault is the usual statement that muscle put into boiling water gives off no CO_2. As a matter of fact it gives off at least 60 c.c. of CO_2 per 100 gms. of muscle and on an average only some 10 % less than the yield from muscle which has undergone heat rigor and subsequent boiling. It yields nearly twice as much CO_2 as a muscle which has only entered heat rigor and has not been heated beyond 45° C.

The hypothesis of 'fixation' of the CO_2 precursor is clearly untenable, but we have to account for the small constant difference between the scalded muscle and the muscle slowly heated to boiling. We know that the muscles after heat rigor, whether subsequently boiled or not, yield about ·4 % lactic acid[1] while those which are dropped into boiling water and escape the heat rigor change, yield less, and sometimes considerably less, than ·1 %. This difference in acidity is quite enough to account for the differences found for the CO_2 discharge in the two cases, on the assumption that we have to do, not with a new formation of CO_2 but with a displacement of preformed CO_2 from its associations in the muscle by means of acid and of heat. If this is so, the addition of acid from outside after the completion of the discharges already described should bring the total discharge of the scalded muscle close to the level of that from the other, and this is found to be the case.

In the experiment given on p. 191, two similar limbs, one heated slowly to 100° and the other rapidly scalded, gave respectively 69·5 c.c. CO_2 % and 59·9 c.c. To each was then added 10 c.c. $\frac{N}{10}$ HCl and the boiling resumed and continued until the whole additional discharge was absorbed for estimation, with the following results:

(*a*) Slowly heated, gave 69·5 c.c. %. Additional yield with acid 28·9 %. Total 98·5 %.
(*b*) Quickly " " 59·9 " " " " 36·0 " " 95·9 "

Another typical example may be given:

(*a*) Slowly heated gave 75·1 c.c. %. Additional yield with acid 31·4 %. Total 106·5 %.
(*b*) Quickly " " 65·4 " " " " 39·3 " " 105·2 "

The question of the origin of the CO_2 which is yielded by the muscle at 100° C. is to be discussed further below. We suggest that the 10 % reduction in the total yield by a muscle so heated, which is found when heat rigor has been avoided by rapidity of heating, is due only to the reduction in such a muscle of the acid available for expulsion of the CO_2.

IV. EXPERIMENTAL MODIFICATIONS OF THE CO_2 DISCHARGES OF HEATED MUSCLE.

Further test of any hypothesis as to the origin of the CO_2 of heat rigor, and of the CO_2 given out at the higher coagulation temperatures, may be found in the effects of previous interference with the muscle upon these yields of CO_2.

---

[1] Fletcher and Hopkins. *Loc. cit.*

§ 1. *The* CO_2 *discharge from heated muscle after survival in oxygen.*

After many hours exposure to an oxygen atmosphere the amphibian muscle, as is now well known, maintains its irritability, shows no approach towards spontaneous rigor, maintains the physical characters of its substance and, while not becoming acid, preserves its normal capacity for producing acid when heated to 40° C. But nevertheless during the exposure it continues to discharge relatively large quantities of CO_2 from its substance[1].

If this large yield of CO_2 in the oxygen atmosphere is derived from the Inogen precursor, then the yield of CO_2 from the muscle when subsequently heated, if that also comes from the same precursor, must be proportionally diminished. If, on the other hand, the CO_2 on heating is only ready formed CO_2 expelled from its associations in the muscle by the rise of temperature and by the accompanying yield of acid, as the results already given have been taken to show, then in spite of long previous exposure to oxygen and indefinite evolution of CO_2 beforehand, the muscle should hold within it still its constant store of CO_2, holding it in the same associations and to the same amount as a freshly excised muscle. Experiment shows that this is actually the case.

The excised limbs were freely suspended in a vertical tubular chamber through which oxygen, freed from traces of CO_2 by soda-lime, and moistened without CO_2 contamination, was drawn, and passed directly afterwards to the baryta absorption chambers. After the exposure to oxygen the limbs were treated for the collection of the heat discharges of CO_2 as already described.

Calculated muscle weights (gms.)	Time of survival in oxygen (hrs.)	Temperature (° C.)	Survival yield of CO_2 in oxygen	Yield at 40—45° C.	Yield at 100°	Total heat discharge (%)
7·0	24	13–14	4·80 c.c. =68·6 %	—	5·26 c.c. =75·2 %	75·2
9·42	24	15–16	12·28 c.c. =130·4 %	3·0 c.c. =32·0 %*	4·50 c.c. =47·8 %	79·8
8·96	48	16–17	22·0 c.c. =245·5 %	—	6·49 c.c. =72·4 %	72·4
8·75	17	11–13	4·07 c.c. =46·5 %	—	6·28 c.c. =71·0 %	71·0
8·79	18	13–15	6·03 c.c. =68·7 %	2·67 c.c. =30·4 %*	3·65 c.c. =41·5 %	71·9

* 3½ hours collection.

In this series it will be seen that the time of survival in oxygen between excision of the muscle and the collection of its heat discharge of CO_2 varied widely in the different cases, and the temperature also varied. Nevertheless, though the total survival discharge of CO_2 in the oxygen atmosphere ranged in the different cases from 46 to 225 c.c. per 100 gms. of muscle, the total CO_2 collected on heating remained very constant, and close to the normal average of about 75 %. Where the discharge for heat rigor at 40 % is separately collected before the temperature is raised to 100° C. it also is found to be normal, at about 35 % of muscle weight. It seems to us to be quite impossible in terms of the 'Inogen' theory, unless wholly unsupported hypothetical extensions of it are to be suggested, to explain how it is that a muscle can still yield the normal amount of CO_2 on entering heat rigor, after having already yielded since its removal from the body more than seven times that amount during survival. The facts are simply explained on the other hand if the heat rigor discharge, here, as in freshly excised muscle, is a discharge of CO_2 already held as such in the muscle tissues.

---

[1] Fletcher. This *Journal*, XXVIII. p. 358. 1902.

The acid production of heat rigor even after 50 hours survival in oxygen is known to be the same as that of freshly excised muscle[1] and must be considered to be available in the same way upon heating for the expulsion of the CO_2 from its positions.

The accelerated discharge in the oxygen atmosphere must be regarded as due to the continued contemporary combustion of some material either easily oxidisable or supplied with suitable oxidases.

This survival oxidation offers many points of interest of which full discussion must be postponed. In passing it may be noted that it is maintained with a remarkable persistence for many hours even when as in the cases given here the muscle is left completely quiescent. For instance, in Exp. 3 of the series just given, the average yield for CO_2 per half hour was found to be maintained at ·19 c.c. for the first 20 hours of exposure to oxygen, ·29 c.c. for the next period of 7 hours, and at ·24 c.c. for the last period of 20 hours. If, as on many other grounds seems probable, this oxidation process is associated with the replacement of lactic acid as it appears, into the molecular position it has just deserted, then we have in the long continued yield of CO_2 a sign and measure of the continued emergence of lactic acid, in the quiescent surviving muscle, and of its incessant oxidative replacement. It may be remembered in this connection that in the experiments which showed that after repeated alternate stimulations and recovery of surviving muscles in oxygen, the final lactic acid yield on heat rigor was the same as that for freshly excised muscle[2], it was found in their course that over a period of many hours it was not the recovery process (diminution of free lactic acid by replacement) but the dissociation due to stimulus (increase of free lactic acid) that declined in extent at successive periods from the beginning. As 'irritability' failed over a period of 50 hours, the yield of lactic acid declined for a constant (external) stimulus, but the oxidative recovery process did not decline in its efficiency to give restoration to a resting level.

---

[1] Fletcher and Hopkins. *Op. cit.*

[2] Fletcher and Hopkins. *Op. cit.* p. 293.

§ 2. *The* CO_2 *discharge from heated muscle after survival without oxygen.*

The effects of previous survival without oxygen after excision upon the yield of CO_2 in heat rigor would be expected to be similar whichever view of the origin of the CO_2 be taken. Upon the 'Inogen' theory, the spontaneous dissociation of CO_2 from a molecule containing intramolecular oxygen and its escape from the muscle by diffusion, would leave a smaller store of the CO_2 precursor for the subsequent instantaneous and final dissociation of heat rigor, and would so far diminish the CO_2 yield of heat rigor. And Hermann found that previous survival, and especially previous contractions, did diminish the subsequent heat rigor yield.

But on the other hand, taking the view we have urged, survival in the absence of oxygen gives slowly, as contractions give quickly, an increase in the dissociated lactic acid within the muscle. In exact proportion as the free acid appears, CO_2 will be expelled from the associations, in the form of carbonates or otherwise, in which it may have been held in the muscle. Any such previous expulsion of CO_2 will so far diminish the amount to be expelled when the maximum acidity of the muscle is attained in heat rigor. The experimental results therefore which are now to be given, though they support those of Hermann, do not necessarily support the interpretation which he and most of his followers have put upon them.

Calculated net muscle weights (gms.)	Time of survival in nitrogen (hrs.)	Temperature (°C.)	Yield of CO_2 in nitrogen	Yield at 40—45° C.	Yield at 100°	Total CO_2 discharge (survival + heating) (%)
11·46	48	16–17	4·8 c.c. =41·9 %	·62 c.c. =5·4 %	3·88 c.c. =33·9 %	81·2
8·71	42	14–16	3·53 c.c. =40·5 %	·69 c.c. =7·9 %	—	—
9·33	24	15–16	2·91 c.c. =31·2 %	—	4·60 c.c. =49·3 %	80·5
6·13	18	17–18	·74 c.c. =12·6 %	·55 c.c. =8·9 %	—	—

The experimental arrangements were the same as in the last section, nitrogen being substituted for oxygen. The nitrogen was passed through strongly alkaline pyrogallate solution for the removal of last traces of oxygen and of CO_2.

In this table of experiments it will be seen that the previous survival of the muscle for long periods in the absence of oxygen produces effects very different in kind upon the two types of CO_2 discharge by heat already described. The discharge for heat rigor at 40° is practically abolished, and only small traces of CO_2 leave the muscle at that temperature. These traces are noticeably smaller, when the muscle has been for longer periods under the anaerobic conditions and has advanced further in the development of acidity and rigor. The increasing acidity will have expelled the normal charge of preformed CO_2 held by the muscle, and little more is left to be expelled by the completion of the acid maximum of the muscle on heating to 40°. On the other hand the discharge at 100° remains approximately normal, and this supports the evidence given in an earlier section to show that the discharge at the higher temperatures is quite different in its nature and origin from the simple expulsive discharge of heat rigor at 40° C.

If the interpretation put on these results is the true one, the total amount of CO_2 which leaves a freshly excised muscle after (*a*) survival in nitrogen, (*b*) heat rigor and (*c*) further heating to 100°, should be equal to the amount leaving a similar muscle which, either just after excision or just after long or short survival in oxygen, is raised through heat rigor to 100° C. This we find to be the case. Two of the experiments already given, after survival in oxygen and in nitrogen respectively, were made with exactly similar muscles *A* and *B*. *A*, during 24 hours in nitrogen gave 2·91 c.c. CO_2 and on being heated to 40° for heat rigor and then to 100° gave 4·60 c.c.; its total discharge after excision was therefore 7·51 c.c. CO_2 or 80·5 °/₀ of muscle weight. *B* was exposed to oxygen for 24 hours, and yielded in that time 12·28 c.c. CO_2 or 130·4 °/₀ of muscle weight. On our hypothesis, however, it was at the end of that time, in spite of this enormous loss of CO_2, still equivalent to a freshly excised muscle, in so far as its capacity for heat discharge was concerned. On being heated to 40° it now yielded 3·0 c.c. CO_2, and on being heated to 100° C. it yielded 4·50 c.c., giving a total heat discharge of 7·50 c.c. or 79·8 °/₀ of muscle weight. This identity of the expulsive discharge in the two cases, as distinct from the combustion or respiratory discharge in presence of oxygen, is very striking, and it has been found whenever for one member of the comparison strictly anaerobic conditions have been secured from excision. If in the anaerobic muscle access of oxygen is allowed at all, either at the beginning of the experiment or later, there will be an increase in the CO_2 yield over and above that due to expulsion by acid formation or expulsion by further heating.

§ 3. *The heat discharge of* CO_2 *after chloroform rigor.*

Exposure to chloroform vapour gives a convenient means of causing rigor rapidly at low (room) temperatures. Chloroform rigor is known to be associated with an increase yield of CO_2[1], of lactic acid[2] and of heat[3]. We find that just as in the case of the muscle in which lactic acid has spontaneously arisen in anaerobic conditions, the chloroformed muscle gives only traces of CO_2 when heated to 40°. And in the same way also, though the heat rigor discharge of CO_2 is practically abolished, the later discharge on heating to 100° is found apparently unchanged in amount. The following examples may be given:

Calculated muscle weight	Conditions of rigor	CO_2 discharge at 40°	CO_2 discharge at 100°
7·64 gms.	Chloroform vapour 24 hrs.	·66 c.c. = 8·7 c.c. %	3·47 c.c. = 45·4 c.c. %
5·95	,, ,, 18 ,,	·95 c.c. = 16 ,, *	2·39 c.c. = 40 ,,
6·9	,, ,, 22 ,,	—	2·45 c.c. = 35 ,,
4·5	,, ,, 7 days	—	1·75 c.c. = 39 ,,
4·42	,, ,, 46 hrs.	·15 c.c. = 3·5 c.c. %	—
3·64	,, ,, 46 ,,	·97 c.c. = 2·7 ,,	—

* Rigor incomplete.

§ 4. *The* CO_2 *discharge after the addition of acid.*

It has been said already that **Pflüger** based his view that the CO_2 of tissue respiration arose by the dissociation of a previously oxidised molecule, as **Hermann** had done, in great part upon the results of heating muscle. The ideas which he gained from a study of the connection between tissue respiration and tissue temperature—a study insufficient of course in itself to decide the question of the mode of origin of CO_2 anaerobically produced—were applied further in the experiments made in his laboratory by **Stintzing**[4]. These experiments were designed expressly to show whether the CO_2 discharged on heating muscle is pre-existing CO_2 held in the muscle, or whether it is through the rise of temperature 'newly engendered' through an increased splitting or dissociation of its precursor.

---

[1] **Fletcher.** This *Journal*, XXIII. p. 50. 1898.

[2] **Fletcher and Hopkins.** *Loc. cit.* p. 265.

[3] **A. V. Hill.** This *Journal*, XLIV. p. 489. 1912.

[4] **Stintzing.** *Pflüger's Archiv*, XVIII. p. 388. 1878.

Stintzing determined the CO_2 discharged from heated mammalian muscle by means of gravimetric estimations of its absorption by soda. His results summarily expressed were these: the muscle heated for some hours at 100° C. gave about 100 c.c. CO_2 °/₀ of muscle weight. This yield was not diminished if the muscle was previously acidified by actual addition of acid, and washed, at 0° C. It was accordingly concluded that the yield of CO_2 was not due to expulsion of pre-existing CO_2 held in the muscle, for this, if any, must have been expelled by the additional acid; but that on the other hand it was the result of a new formation of CO_2 by a splitting process accelerated by heat. This CO_2 newly dissociated from a previously oxidised molecule was considered to arise at the high temperatures faster but in no different manner from that coming from the muscle at the normal temperatures, spontaneously after excision, or more quickly as the result of stimulation.

Very grave fallacies however underlie these results and conclusions. In the first place the muscle (chiefly that of the hind limbs of rabbit) was cut up by a mincing machine in the open to provide material for the experiments. It is known that mammalian muscle so treated reaches its maximum acidity with extreme rapidity[1], and that this development of acid is accompanied by a large discharge of CO_2, which very rapidly escapes[2]. In some cases, but not in all, **Stintzing** froze the muscle before mincing it, but not enough detail of procedure is given to indicate how far this was likely to be effective. After mincing, the muscle fragments were boiled in water, and the discharged CO_2 collected. It is not surprising that the results after variable treatment beforehand differed widely. The totals given range from 50 °/₀ to 168 °/₀ of muscle weight. **Stintzing** gives the average as 100 °/₀, but of the nine experiments quoted, four give an average of 135 °/₀, while the other five give 70 °/₀.

To the chopped muscle, in another series, phosphoric acid (or sulphuric acid) was added in the cold (0–5° C.) for a few minutes, or for an hour or two, the muscle was washed with distilled water, and then boiled as before. In the five cases quoted the CO_2 discharge on boiling the muscle lay between the limits 68 and 83 °/₀ of the muscle weight—all decidedly below the average 100 °/₀ quoted for the untreated muscle. But **Stintzing** argues that as 'not much less' was yielded by the acidified muscle, the chief part of the CO_2 collected cannot have been held as such in the muscle.

---

[1] **Fletcher.** This *Journal*, XLIII. p. 286. 1911.

[2] **Fletcher.** *Ibid.* XLVII. p. 361. 1913.

The rest of his experiments show that previous digestion of the muscle at 40° for 22 hours, and previous tetanus, alike gave a diminution of the CO_2 yield on boiling to 30–35%. This can be explained, as we have pointed out already, upon either hypothesis, as due to diminution of a store of CO_2 precursor by previous demands upon it, or as a diminution of the normal holding of preformed CO_2 due to a previous partial expulsion by acid formed in the muscle.

So far as the experiments with acid go, Stintzing's own figures show an undoubted reduction of the heat discharge after acidification, and so far point to an expulsion of preexisting CO_2. But as he took them in the contrary sense, and they have so often been quoted as supporting the theory of intra-molecular oxygen, we have made experiments of the same kind ourselves.

With amphibian muscle the danger of rapid rigor accelerated by mincing, the danger, that is, of producing a change before the experiment begins which is to investigate it, can be avoided. In the series of estimations to be given the limbs were placed in the flask as before and 50 c.c. $\frac{N}{10}$ HCl added, with 1·5% NaCl dissolved in it. The addition of the salt prevented excessive swelling of the muscle in the acid and avoided the effects of mechanical injury. After addition of the acid salt solution the flask was immediately closed and the current started. Evolution of CO_2 always began at once and the discharge was collected in the usual absorption tubes. After cessation of the discharge, fresh absorption tubes were introduced and the unopened flask heated to 40°. It was invariably found that the normal heat rigor discharge was greatly reduced, or represented only by traces of CO_2. On the other hand, when again the temperature was raised to 100° C. after completion of the 40° yield, a normal yield of CO_2 was obtained without reduction. This is shown in the following table, p. 200.

These results not only show a great reduction, or virtual abolition, of the heat rigor discharge after previous acidification for the expulsion of preformed CO_2, even when the heating takes place in the presence of the acid, but they show that while the heat rigor discharge is reduced to a minimum, the discharge at 100° remains unaffected. Here again the complete difference between the one discharge and the other is established, and the facts explain how it was that Stintzing, who did not distinguish between the discharge at 100° and that for heat rigor

	Limb weights, gms.	Calculated muscle weights	Previous treatment with acid	CO_2 yield at 40° C.	CO_2 yield at 100° C.
*	8·55	7·12	44 hrs. at 13–15° C. (CO_2 discharge not collected)	·39 c.c. = 5·5 %	—
*	8·50	7·08	" " "	—	3·35 c.c. = 47·3 %
	8·8	7·33	24 hrs. at 15–17° C., giving 8·42 c.c. CO_2 = 114·9 %	·52 c.c. = 7·1 %	3·22 c.c. = 43·9 %
	9·65	8·04	46 hrs. at 15–17° C., giving 13·47 c.c. CO_2 = 167·5 %	—	3·63 c.c. = 45·1 %
	11·33	9·44	48 hrs. at 0–5° C., giving 8·78 c.c. CO_2 = 93·2 %	·68 c.c. = 7·2 %	3·12 c.c. = 33·1 %
	5·43	4·53	48 hrs. at 0–5° C., giving 4·54 c.c. CO_2 = 100 %	—	1·71 c.c. = 37·7 %

* Crossed limbs.

alone, found only a partial diminution of the boiling yield after acidification. A partial diminution he did find, but he made so little of it that his experiments are commonly quoted as proving that there was none.

It will be seen that in the experiments in which the acid was acting upon the muscle maintained at low temperature (0–5° C.) considerable quantities of CO_2 were expelled—in the two cases quoted, 93 c.c.% and 100 c.c.% of muscle weight. Hermann, it may be noted, asserted that muscle to which phosphoric acid was added at 0° C. yielded no CO_2 at all, and no doubt that belief discouraged him from recognising preexistent CO_2 in the muscle. We consider the CO_2 yielded in our experiments to be preexistent CO_2 expelled by the strong acid from its combinations in the muscle. Upon the view of Hermann or Pflüger however it is regarded as dissociated CO_2 from the CO_2 precursor. But this precursor, the same, according to Hermann, as the lactic acid precursor, does not tend to dissociate at 0–5° C. even when subjected to extreme mechanical violence[1]. It seemed possible that the acid might fail also to dissociate the lactic acid precursor at that temperature, and if it did, while at the same time it yielded CO_2, this would be strong additional evidence that the CO_2 resulted from simple expulsion and not from dissociation, and would at least prove that the lactic acid and CO_2 did not arise simultaneously from the dissociation of a common labile molecule. Samples of muscle treated with acid in this way were accordingly treated for lactic acid estimation.

Ten pairs of freshly excised undamaged limbs were weighed and immersed in 300 c.c. N/10 HCl previously cooled to 0–2° C. to which 4·5 gms. NaCl had been added. The limbs remained in the acid, maintained close to 0° C. throughout, for 24 hours. Sodium bicarbonate to the amount calculated for exact neutralisation of the hydrochloric acid was then added. Neutralisation was reached in an hour or two. The muscles were then removed and ground up in ice-cold alcohol for estimation in the ordinary way. The liquid was taken down to dryness, the residue extracted with alcohol and the alcoholic extract added to that of the muscle. In other cases quoted below, two similar sets of ten limbs were taken (after 'shuffling' for average equality) and treated with acid in the cold as described already. One was then extracted with ice-cold alcohol, while the other limbs, still under acid, were brought to 40° C. and kept at 40° for an hour, before extraction. The estimations of lactic acid by the Fletcher and Hopkins method were very kindly undertaken for us by Mr Winfield, to whom we are greatly obliged.

---

[1] Fletcher and Hopkins. *Op. cit.*

The results of the estimations were as follows.

	Zinc lactate
68·4 gms. of muscle (net weight), after 24 hours in acid at 0–2° C. yielded	·126 %
68·2 „ „ „ „ „ „ „	·162 %
65·9 gms. of muscle (net weight) similarly treated with acid in the cold but subsequently raised for 4 hours to 35° C. yielded	·241 %
47·5 gms. (weight calculated from limb weights) treated with acid in the cold for 1 hour and subsequently raised for 4 hours to 40° C. ...	·236 %

These low figures for the lactic acid yield by the muscles kept in the acid salt solution at 0–2° C. are probably to be taken with fairness as representing the resting 'minimum.' For quite fresh undamaged muscle Fletcher and Hopkins found the yield to be raised by the inevitable manipulations from what may be assumed to be zero to ·05 or ·1 %. The figures given above, ·12 and ·16 %, are little above this resting level, and far below the constant maximum figures of ·4–·5 % found by Fletcher and Hopkins for the yield by muscle after heat rigor or severe and complete mechanical injury. Yet while the lactic acid yield remains so low, the volume of CO_2 expelled from the muscle in the cold acid may, as we have shown, be double or treble the amount discharged during heat rigor.

If, after long treatment with the acid salt solution in the cold, the muscle while still in the acid solution be heated to 35–40°, the lactic acid yield is increased. The yield (about ·24 %) at this rigor temperature after long treatment with acid is not equal to the maximum obtained in the heat rigor of untreated muscle, and this raises some interesting questions with regard to the nature of the lactic acid production itself which we hope to deal with later. But while heating the long acidified muscle to 40° does give an increase of lactic acid yield, it gives only traces of CO_2, as we have seen previously. The relationships of the yields of lactic acid and CO_2 respectively are in fact exactly inverted at the two temperatures, 0° and 40°, and this appears to us again to forbid the view that the CO_2 and lactic acid arise by simultaneous or successive dissociation of a common precursor like that postulated in the Inogen theory.

SUMMARY OF RESULTS AND CONCLUSIONS.

By volumetric estimation of their absorption in baryta solution the total amounts of CO_2 discharged by excised amphibian (frog) muscle when heated have been determined.

(1) The total discharge accompanying heat rigor induced at 40° C. amounts to 35–40 c.c. CO_2 per 100 gms. of the net muscle weight.

(2) This discharge associated with heat rigor does not, as Hermann and other subsequent writers have supposed, exhaust the capacity of the muscle for yielding CO_2 on further heating. After completion of the CO_2 discharge for heat rigor, the muscle yields a further 35–40 c.c. CO_2 °/₀ on being heated to 100° C. Of this additional discharge 15–20 °/₀, and not more, is obtained on heating to 75° C. for the complete heat-coagulation of the proteins. A further 15–20 °/₀ is yielded only when the temperature is close to 100° C.

The excised muscle accordingly, when it is heated so as to enter complete heat rigor at 40° C., and also further heated to 100° C., yields a total of 70–80 c.c. CO_2 per 100 gms. of muscle.

(3) If the muscle on the other hand is 'scalded' or brought as rapidly as possible to 100° C. by sudden immersion in boiling salt solution, it does not become acid by lactic acid formation (as du Bois Reymond, confirmed by Fletcher and Hopkins, showed), but it discharges a considerable quantity of CO_2. Such a scalded muscle yields 60–70 c.c. CO_2 per 100 grms. of muscle, or only about 10 °/₀ less than a muscle slowly heated to 100° C., and this is in contradiction to the statement of Hermann, which is still very commonly repeated, that muscle plunged in boiling water yields no CO_2 at all.

(4) Excised muscle which has been exposed for a few or for many hours to an atmosphere of oxygen, exhibits the same normal discharges of CO_2, whether in heat rigor at 40° C. or on further heating to 100° C. It behaves in these respects exactly like freshly excised muscle, even though it may have lost previously during its survival in oxygen several times as much CO_2 as that of the whole of the normal heat discharges combined.

On the other hand, previous survival in an anaerobic atmosphere (nitrogen), reduces markedly, and if the muscle has been several hours without oxygen virtually abolishes, the CO_2 yield at 40° C., though it does not appear to affect the discharge on heating to 100° C., and this remains normal.

The total CO_2 given off during many hours to the anaerobic atmosphere by the muscle as it becomes increasingly acid, together with the reduced amount subsequently obtained from the muscle in heat-rigor, and its discharge, not reduced, on subsequent heating to 100°, is closely equal in sum to the amount yielded by a similar muscle during heat-rigor and heating to 100° alone, either immediately after excision, or after a few or very many hours previous exposure to oxygen.

(5) A muscle which has entered chloroform rigor, like the muscle after long deprivation of oxygen, yields only traces of CO_2 at 40° C., but gives its normal discharge at 100° C.

(6) If acid is added to the muscle at room temperatures or at 0–5° C., a large yield of CO_2 (over 100 %) leaves the muscle. Subsequent heating to 40° C. gives only traces of CO_2; while further heating to 100° C. gives approximately normal amounts.

If acid is added to the muscle at temperatures close to 0° C., while a discharge of CO_2 greater than that for heat rigor leaves the muscle, the yield on the other hand of lactic acid even after many hours is very little increased above that found for fresh resting muscle. If the acidified muscle after the escape of the CO_2 be now heated to 40°, the lactic acid yield is increased (though it does not reach the normal heat maximum for untreated muscle) while, as already stated, only traces of CO_2 are discharged.

It is concluded that the CO_2 which is discharged from normal excised muscle during heat rigor induced at 40° C., is preexistent CO_2 expelled from its combinations as carbonate or otherwise by the acid which is known to be set free in the muscle in heat rigor. The discharge of CO_2 on the other hand which leaves the muscle at the higher temperatures, 75° and 100°, is here regarded as CO_2 which is held by muscle colloids, or in combination with amino-acid groups, and it is suggested that this is expelled in part when the physical changes of heat-coagulation occur, and in part on the dissociation by heat of carbamino-groups or other combinations of protein constituents with carbonic acid.

Any circumstances which increase the acidity of the muscle beforehand (*e.g.* anaerobic survival, the action of chloroform, or the addition of acid from outside) will be expected to expel the CO_2 which would otherwise be liable to expulsion during the formation of acid in heat rigor, and so proportionately to reduce, as they do reduce, the CO_2 yield of subsequent heat rigor. But the same previous treatment of the muscle would not necessarily be expected to influence, and does not markedly influence, the CO_2 discharges at the higher temperatures.

Reasons have been given against accepting the experimental results of heating muscle obtained by Hermann, or by Pflüger and Stintzing. The Inogen theory, with its assumption of 'intramolecular' oxygen, based in great part upon these observations of the CO_2 discharges from heated muscle, is not supported by our own results. What we have found in examining these discharges in different circumstances agrees well with the results obtained already in this laboratory, by the study of surviving muscle in regard to its production of CO_2, of lactic acid and of heat, and supports the view that it is only in the presence of available oxygen that fresh production of CO_2 takes place. Without available oxygen CO_2 may indeed leave the muscle, and so appear to be produced, but this happens exactly in those conditions in which lactic acid is known to be accumulating in the muscle, and such a discharge of CO_2, increased whenever the conditions for lactic acid formation are made more favourable, is most easily to be explained as an expulsion of CO_2 by the acid from the temporary combinations in which it is held in the muscle. The amount of CO_2 so held in the muscle will be at its greatest during and just after a free supply of oxygen for the oxidative processes yielding CO_2, and when the supply of oxygen is withheld it will be diminished in proportion as the acidity of the muscle increases.

All the facts hitherto advanced in support of the Inogen theory, according to which the CO_2 and lactic acid were supposed to arise from a common precursor previously oxidised, can be explained in terms of this acid expulsion of temporarily combined CO_2, while on the other hand the experimental results given here are not explicable if the Inogen theory is held. The inverted relationships of the lactic acid and CO_2 productions at 0° and 40° respectively which have been described are taken as further evidence against their supposed origin from a common source. All the evidence gained in this laboratory in recent years points to the conclusion that in the muscle the respiratory oxidative process yielding CO_2 as an immediate product has its chief end in the supply of energy for replacing the lactic acid in the molecular position from which stimulation of some kind has displaced it, and there appears to be no reason at present for supposing that the material which is oxidised in the respiratory process is the same as, or related to, the material from which lactic acid appears and into which, as it seems, it may again disappear.

OBSERVATIONS UPON DECEREBRATE RIGIDITY. By

LEWIS H. WEED, M.D., *A. T. Cabot Fellow in Charge of the Laboratory of Surgical Research, Harvard Medical School.*

(*From the Physiological Laboratory of the University of Liverpool.*)

SYNOPSIS.

PAGE
I. Relation of cerebellar cortex to decerebrate rigidity . 208
II. Effect of various cerebellar ablations upon decerebrate rigidity 211
III. The pathway in the cerebellar peduncles . . . 214
IV. Mesencephalic reactions in decerebrate rigidity . . 215
V. The inhibitory pathway 218
VI. Abnormal reactions 221
VII. Summary and evidence for existence of probable pathway of reflex 222

Introductory. The condition of prolonged contraction in the extensor muscle groups, supervening upon removal of the cerebral hemispheres and the basal ganglia, was first described by Sherrington[1] and designated "decerebrate rigidity." The manifestation of this state of enduring muscular contraction seems to warrant further study, not only because of the physiological interest attached, but also because of the likelihood, as first suggested by Hughlings Jackson, that these experimental rigidities have great similarity to the clinical rigidities, observed in the meningitides, etc. It was hoped that diagnostic importance might eventually be attached to the occurrence of a rigidity clinically, provided a definite pathway for the reflex could be established.

Decerebrate rigidity manifests itself as a prolonged, enduring contraction of the extensor muscles. The forelegs are stiffly extended at the elbow and the shoulder is fixed in a retracted position. The knee, hip, and ankle also exhibit marked extension. The neck shows the contraction in the retractors of the head, while the tail is carried dorsally in extension, frequently in degree sufficient to sustain its own weight. The rigidity is observed ordinarily first in the elbows and then in the knees—the two joints which ultimately show in most extreme measure the contraction. Very quickly after the involvement of elbows and knees, the shoulders and hips become affected. Later, the ankle and wrist are involved, the ankle usually in greater measure than the wrist. In the dog, the involvement of the wrist in the extensor rigidity is invariable, but in the cat this joint seems in practically all cases to be unaffected. Individual variations may exist, but the main features of this extensor rigidity in the various body joints are common to practically all of the animals used for experimental purposes. Sherrington considers it to be essentially the reflex posture of standing[1].

---

[1] Sherrington. *Proc. Roy. Soc.* LX. p. 414. 1896; *Journ. Physiol.* XXII. p. 319. 1898; see also, Lowenthal and Horsley, *Proc. Roy. Soc.* LXI. p. 20. 1897.

Customarily, the decerebration of these animals is done by a single cut through the midbrain by means of a blunt knife or spatula. The midbrain is transected just cephalad to the superior colliculi permitting the removal of hemispheres and basilar nuclei. Respiration may be momentarily suspended, but spontaneous breathing is usually resumed after artificial inflation of the lungs has been maintained for a short time. The extensor rigidity becomes apparent in the elbow, ordinarily within two to five minutes after the decerebration, although its onset may be somewhat delayed by the depth of narcosis or, as occurred in one of these observations, by pressure on the vertebral arteries. Shortly after the rigidity becomes appreciable in the elbow, it involves the other joints. Ten minutes after decerebration, there is present a fair degree of rigidity, but this gradually increases during the next hour and a half to the ordinary maximum observable in these animals, unless other procedures to diminish or to augment the rigidity have been instituted. This customary degree is maintained without change for many hours after it has reached its maximum.

Considerable work, furthering our knowledge of decerebrate rigidity, has already been done. Sherrington[2] has shown that these prolonged contractions are dependent, as is normal tonus, upon the inflow of afferent impulses from the limb, for section of the posterior spinal roots abolishes the rigidity at once. Division of the posterior columns of the spinal cord, however, has no influence upon its existence. A cut made in the ventro-lateral portion of the thoracic spinal cord destroys the rigidity in the ipsilateral hind limb. That the source of the rigidity is not the pyramidal tract is shown by its disappearance ipsilaterally after a hemisection of the bulb above the decussatio pyramidum. The rigidities, according to Sherrington, sometimes persist after removal of the cerebellum. Removal of one cerebral hemisphere causes the development, in most cases, of an ipsilateral rigidity. This unilateral development of rigidity is by no means as invariable as after ablation of both hemispheres, but the ipsilateral character of the rigidity, when it did develop after unilateral ablation, suggested a possible cerebellar pathway for the impulse. There are, in the literature, no suggestions as to the possible origin of the motor impulses causing the development of these prolonged extensions.

---

[1] Sherrington. This *Journal*, XL. p. 103. 1910.

[2] *Loc. cit.*

Methods of Investigation. This present work was done entirely on cats, the series comprising about 50 animals. Full anæsthesia was maintained until decerebration had been performed. The carotid arteries were both ligated and a T-tube introduced into the trachea. The skull was trephined beneath the temporal muscle. The dura was opened and with the deepest possible anæsthesia the midbrain was transected with a blunt instrument. The hemispheres and basilar nuclei were removed through the temporal opening, and the bleeding controlled by pressure upon the vertebral arteries. After decerebration, the animals were suspended in a comfortable position, care being taken to keep this factor constant throughout the series of observations.

The decerebration was, in many of the experiments, preceded by various operative measures, especially those executed with regard to the possible relation of the cerebellum to the development and maintenance of the rigidities. In three cats, extirpations of the whole or portions of the cerebellum were made under aseptic precautions and the animals observed for various periods after the operative procedure.

In the majority of the cases, the animals were left undisturbed for approximately 90 minutes after decerebration (at which time a constant degree of rigidity will have developed); extirpations of various parts of the cerebellum and of the midbrain were then made. No anæsthesia was necessary in these secondary operative manipulations, since consciousness vanished with removal of the cerebral hemispheres. Observations of the degree of rigidity were taken at frequent intervals through the course of the experiment. It was deemed essential to treat each case as an individual, presenting comparative degrees of rigidity rather than absolute degrees, but after some experience in the study of these rigidities, it was found that an absolute degree of rigidity could be established with but little variation from animal to animal. The maintenance of a constant method of suspension in any study of rigidity was considered necessary, for **Ewald**[1] has shown the close relation of the semi-circular canals to normal tonus.

The degree of rigidity was estimated chiefly by the amount of pressure required to overcome the enduring muscular contractions. This can best be done in the forelegs by placing the fingers of one hand upon the vertebral border of the scapula and pressing with the fingers of the other hand upon the paw. In addition to the resistance offered by the rigid muscles to this passive articular movement, much reliance could be placed upon the position of the limb, upon the degree of flexion at elbow and knee, and upon the amount of fixation at shoulder and hip. The position of the tail and the resistance offered to passive opening of the mouth are also of importance. In this work, but little regard was bestowed upon the retraction of the neck because of the frequent freeing of the posterior neck muscles from the occipital bone in the cerebellar exposures. This retraction of the neck is, however, a valuable index in those cases in which the muscles moving the head are undisturbed.

In all of these observations, no reliance was placed upon the results unless the animal continued to breath spontaneously throughout. The degree of rigidity in many cases is maintained without change under artificial respiration, but with failure of spontaneous breathing there is great likelihood that the mesencephalon is likewise unresponsive to normal stimuli. With the development of this work, the necessity of ensuring a responsive midbrain became absolute.

The operative procedures employed about the cerebellum were, for the most part, done with blunt instruments, especially the ablations. Every possible care was taken to avoid injury to the vestibular mechanism. No marked hæmorrhage, sufficient to vitiate in any way the results, was occasioned.

I. RELATION OF CEREBELLAR CORTEX TO DECEREBRATE RIGIDITY.

These observations were undertaken with the view that probably the cerebellum presented an essential link in the reflex which occasions decerebrate rigidity. In the earliest of these experiments, the first procedure consisted in the removal, under complete anæsthesia, of the bone over the posterior portion of the cerebellum, the opening of the cerebellar dura, and subsequent decerebration within a few moments. In only one of these animals did any rigidity develop and then only for a minute or so, very quickly after the transection of the midbrain. In the other animals, however, no rigidity at all appeared—the animals continuing to show throughout the period of observation an attitude of flaccid flexion. This failure to develop rigidity, even after several hours, suggested that the cooling and unavoidable trauma applied to the cerebellar cortex during the removal of the overlying bone were sufficient to interrupt the essential pathway. In view of Edinger's[1] and of Horsley and Clarke's[2] conception of the afferent (as regards cerebellum) function of the cerebellar cortex, the idea that the link was represented in the cortex of the cerebellum seemed more than likely.

---

[1] **Ewald.** *Nervus Octavus*, Wiesbaden, 1892.

Were this hypothesis of an essential cortical function for the cerebellum in the development and maintenance of decerebrate rigidity to be subjected to absolute proof, destruction of the available cerebellar cortex by means of scraping or of maceration should prove to be equally efficacious in preventing the development of the rigidity after subsequent decerebration. To place this supposition on an experimental basis, the cerebellar cortex, so far as it could be exposed posteriorly, was destroyed and decerebration was then done. An extensor rigidity of regular degree developed in the customary time and the animal exhibited, throughout the period of observation, the characteristic phenomena of any decerebrate preparation. It must be kept in mind, however, that only a small part of the cerebellar cortex can be destroyed through the posterior exposure, as the whole anterior cortex (lying beneath the bony tentorium), the anterior portions of the lateral lobes, and the whole inferior vermis remain intact. Then, too, the difficulty of destroying, even with sufficient exposure, all of the cerebellar cortex lying over the ramifications of the arbor vitæ, is insurmountable. Cold and fluids should prove much more efficient in effecting such a paralysis of the cerebellar cortex.

Consideration of the divergent results already obtained from exposure and destruction of the posterior cerebellar cortex made it seem likely that exposure of the cortex, after decerebration with subsequent development of extensor rigidity, should prove to have no influence upon the course of the rigidity. This has been verified in very numerous observations—the only phenomenon occasionally noticed is a marked accentuation of the rigidity during the operative procedure incident upon removal of the bone over the cerebellum. This accentuation lasts but a few moments and the animal then resumes its ordinary degree of extensor rigidity unless further procedures about the cerebellum are instituted. Considering this momentary accentuation of the rigidity during the removal of the bone as a probable stimulation due to traumatic manipulations, it does not seem at all strange that a hot saline swab (*circ.* 45°) applied to this posterior cerebellar cortex should occasion a short-enduring but very marked increase in the existent rigidity. The converse of this observation, diminution of the rigidity by the application to the same area of a cool saline swab (*circ.* 16°), has been also confirmed in several experiments. Similarly, the application of a 1 °/₀ solution of cocaine hydrochloride to the same areas causes a diminution of the rigidity, but it hardly appears to be as efficacious as the cool saline. The application of cool saline or of the cocaine solution to the cortex of the superior vermis alone, effects only a slight reduction in the rigidities in the various extremities, but the diminution in rigidity is more readily appreciated in the neck and tail.

---

[1] Edinger. *Nervöse Zentralorgane*, I. Leipzig.

[2] Horsley and Clarke. *Brain*, XXXI. pp. 45–124. 1908.

Believing that possibly there existed in the cerebellar cortex some localisation for the rigidities in the extremities, the posterior cortex was exposed on one side only. Subsequent decerebration was followed by the development of a bilateral extensor rigidity, less prompt in appearance and slightly milder in degree on the ipsilateral than on the contralateral side. Similar results were obtained after destruction of the posterior cortex on one side. The superior vermis also seems to have especial representation of the trunk and tail areas.

In these observations, many attempts have been made without success to find areas in the cerebellar cortex, which would, under unipolar excitation, have an augmenting effect on the existent rigidities. However, we have been able to confirm repeatedly the inhibiting area localised by Sherrington[1] and also by Lowenthal and Horsley[2] in the anterior part of the superior vermis. Stigmatic stimulation of this area causes an immediate marked inhibition of the rigidity, equal in effect on both sides. After cessation of the excitation, the extensor rigidity very quickly returns. Apparently, in the cat, the inhibitory area is fairly well delimited to the superior vermis.

The cerebellar cortex, as far as it may be exposed in the cat, possesses, then, some definite relationship to decerebrate rigidity, but undoubtedly the function is double—an excitatory and inhibitory link both existing. The technical difficulties have been too great to ascertain exactly the effect of a complete cortical extirpation, leaving intact basilar cerebellar nuclei.

---

[1] *Proc. Roy. Soc.* LX. 382. 1896.

[2] *Ibid.* LXI. p. 20. 1897.

II. EFFECT OF VARIOUS CEREBELLAR ABLATIONS UPON DECEREBRATE RIGIDITY.

(*a*) *Removal of lateral lobe of cerebellum.* The exposure and ablation of one lateral lobe of the cerebellum, leaving the vermis region covered by dura and bone and avoiding injury to the vestibular complex, result in diminution of the rigidity in the ipsilateral fore and hind legs. The rigidity of the trunk and of the tail remains unaffected. There persists, however, some degree of extensor rigidity in the ipsilateral limbs while the contralateral extremities show the customary marked rigidity. This result is in accordance with an accepted view of cerebellar localisation (*i.e.*, the vermis represents tail, trunk, and head, while the lateral lobes are concerned with the four legs) but it also shows how relatively diffuse the cerebellar localisation is in regard to the occurrence of definite areas for the peripheral rigidities.

(*b*) *Removal of one-half of cerebellum.* In a number of cats, after decerebration with the development of the customary degree of extensor rigidity, one-half of the cerebellum has been exposed and extirpated. The majority of these observations showed a total loss of rigidity in the ipsilateral limbs, the fore and hind legs lying in a flaccid flexor attitude. In this procedure, one-half of the vermis was also extirpated and, as an apparent consequence, the tail showed diminution of its customary dorsal flexion. In two animals in this series, the ablation of one-half of the cerebellum was not followed by the enduring total loss of ipsilateral rigidity. In one, the rigidity was merely diminished and did not disappear until the remainder of the vermis was removed. In the other cat, the rigidity on the same side disappeared for a short time and then returned, to maintain as great a degree of rigidity as on the opposite side.

If, on the other hand, one-half of the cerebellum be removed before decerebration, extensor rigidity develops, according to these observations, on both sides. However, in the ipsilateral limbs the rigidity was slightly less in degree than in the contralateral legs; the foreleg on the same side showed greater flexion at the elbow and passive motion required less power.

(*c*) *Removal of vermis region.* The removal of the whole vermis in a cat, exposing the ventricular floor beneath, did not affect the development of a bilateral extensor rigidity of the customary degree which ensued upon the subsequent removal of the cerebral hemispheres. The tail in this case showed a very marked degree of dorsal flexion.

In a number of animals the cortex of the anterior portion of the superior vermis has been extirpated. The limits of the area removed corresponded to the limits of the inhibitory centre mentioned above. In every case, the manipulative procedure occasioned a brief diminution in the existent rigidity immediately after the extirpation, but this was always followed by the development of a very extreme degree of extensor rigidity, the forelegs and hind legs showing absolutely no flexion at elbow and knee respectively. It is almost impossible to overcome by passive pressure the rigidity which ensues after such an extirpation.

(*d*) *Removal of the whole cerebellum.* In a considerable number of cats, the whole cerebellum has been removed at an interval after the initial procedure of decerebration and after the development of a constant degree of extensor rigidity. Spontaneous respiration has continued in these animals without interruption, often for several hours. Care has been taken to avoid injury either to the peripheral vestibular pathway or to its central nuclei. This may be easily done by elevation of the whole cerebellum away from the ventricular floor, with subsequent section of the peduncles laterally.

The results of these total ablations of the cerebellum have been in entire agreement except in one instance. Section of the cerebellar peduncles is always followed by a marked accentuation of the existent rigidity, as was remarked by Thiele[1]. Immediately after this accentuation, if the whole cerebellum be removed, the rigidity begins to diminish rapidly. In all of these cases, except in the one instance cited, the rigidity entirely disappeared leaving the animal in a condition of flaccid flexion. The time required in these acute experiments for the disappearance of all traces of the extensor rigidity varies considerably, but ordinarily it is gone within twenty minutes after the cerebellar extirpation. In some cases, the total loss of rigidity has occurred within five minutes after the removal of the cerebellum; in one other extreme case it persisted for forty minutes before totally disappearing. The variability in the time required for the disappearance of the rigidity does not appear extraordinary when one considers the inevitability of a fusion of stimulation and paralysis which ensues after such an extirpation. In one of these cases, the animal remained for several hours in an attitude of flaccid flexion, breathing spontaneously: at the end of this time the flaccid flexion developed gradually into a definite flexor tonus of mild degree.

---

[1] *Journ. of Physiol.* XXXII. p. 358. 1905.

The one exception to this series of experiments consists in the persistence of a decerebrate extensor rigidity after the secondary complete removal of the cerebellum. In this cat, decerebration was performed, and after awaiting the acquirement of the constant degree of rigidity, the calvarium was removed and the cut surface of the midbrain stimulated by hot swabs and by the unipolar electrode. The whole cerebellum was then removed, in order that comparison of the mesencephalic reactions, before and after the cerebellar ablation, might be made. Immediately after the ablation, the midbrain was repeatedly stimulated. But contrary to expectation, the rigidities did not disappear in the course of the next twenty minutes, but they persisted for over an hour. Whether the mesencephalic manipulations were the cause of the persistence of the rigidity or not, is doubtful: the observation remains an isolated case of persistence of the decerebrate rigidity after removal of the cerebellum.

The observations recorded above were all made as acute experiments, and there remains to record the results of decerebration in a cat in which total ablation of the cerebellum had been done some four weeks previously. This animal made a rather slow but perfect recovery from the shock of the cerebellar ablation and was in very good condition at the time of decerebration. During the first few days after the operation, the animal showed marked extensor rigidity in the fore and hind limbs, equal on both sides. This disappeared and did not reappear. There was a suggestive ocular ataxia at times, shortly after the extirpation, but it was never a definite nystagmus. Throughout, the animal showed the grossest kind of ataxia in all movements, a rather marked tremor, inability to stand (apparently due to the ataxia) and, for the first two weeks, marked asthenia. This weakness passed away, however, rather quickly after the first fortnight following the extirpation. The effect of this early removal of the cerebellum upon the subsequent development of decerebrate rigidity is, of course, highly important to a true conception of the process.

In this decerebellate animal, decerebration was followed within two or three minutes by the development of extensor rigidity in the elbows and knees, equal on both sides. This rigidity increased and in the course of the next ten minutes attained the normal degree for such a period after decerebration. The elbow, shoulder, hip, knee and ankle were involved on both sides. The knee jerks were equal, brisk and showed the delayed relaxation that characterizes the reflex in decerebrate animals. The rigidity was everywhere extensor in type, the tail showing marked dorsal flexion and the neck considerable retraction. It involved, of course, the same joints in the usual manner as in the ordinary decerebrate preparation, but in some inexplicable way it seemed to lack the customary plasticity of the regular decerebrate animal. However, after the development of this customary degree of decerebrate rigidity in the first few minutes, in place of the regular increase during the next hour, there was a very considerable diminution of the degree of extensor rigidity. One half-hour after decerebration, there still existed a very mild degree of extensor rigidity, everywhere apparent in the customary joints. This mild form underwent, very slowly, a further reduction, the rigidity being of just appreciable degree two hours and thirty minutes after decerebration. Two hours and thirty-five minutes after decerebration, the rigidity had entirely disappeared and the animal exhibited everywhere an attitude of flaccid flexion. The cat continued to breathe spontaneously for an hour and a half longer, the flaccid flexor attitude persisting without the reappearance of any rigidity until the death of the animal.

An acute observation, similar in outline to the above, was also made but with different results. In this, the cerebellum was first removed and immediately thereafter the animal was decerebrated. Very quickly after the decerebration, a mild degree of rigidity appeared and persisted only for one or two minutes. Then it rapidly disappeared, leaving the animal in a condition of flaccid flexion. During the remainder of the experiment (five hours), no rigidity of any type made its appearance, the relaxed flexor attitude being characteristic of the animal.

III. THE PATHWAY IN THE CEREBELLAR PEDUNCLES.

With the growth of the conception that the cerebellum possesses an important, if not essential, pathway for the development and maintenance of decerebrate rigidity, it became imperative to determine by which peduncles the impulses are carried to and from the cerebellum. Section of the various peduncles can be done quite easily in the cat, provided that the ultimate recovery of the animal is not intended. The inferior peduncles can be approached in the midline posteriorly, while the middle peduncles are easily accessible if the lateral lobes of the cerebellum be elevated. In a decerebrate animal, the superior peduncles can be dealt with from above, after removal of the bony tentorium. The divisions of the peduncles made in these observations were subsequently confirmed by examination post mortem. Practically all of these peduncular sections have been made on animals in whom decerebration was the first step.

In these observations, section of both of the inferior cerebellar peduncles (corpora restiformia) and of the middle cerebellar peduncles (brachia pontis) has had absolutely no appreciable effect on the existent extensor rigidity. Section of both superior cerebellar peduncles (brachia conjunctiva) results in a marked initial accentuation of the existent rigidity, followed by a rapid and complete loss of all the rigidity. The animal shows the characteristic attitude of flaccid flexion, without any appreciable tonus. Section of one superior peduncle results in an initial augmentation of the rigidity on the same side, followed by a marked diminution of the ipsilateral rigidity but never a total loss. The rigidity of the contralateral extremities is apparently not affected.

In one animal, an attempt to section both superior cerebellar peduncles before decerebration was made. As confirmed by later investigation, only a portion of both peduncles was sectioned; it is interesting, however, in view of the fact that the extensor rigidity which followed removal of the cerebral hemispheres was very mild in degree and not at all comparable to that usually obtained. Subsequent section of the remaining portions of the superior peduncles resulted in the abolition of all rigidity.

In another animal, all of the three peduncles on one side were sectioned, and on the other side, the inferior and middle peduncles were completely severed as well as a portion of the superior. Marked initial accentuation of the existent rigidity occurred, followed quickly by complete loss of all rigidity on the side on which all the peduncles were sectioned; on the other side only a very mild degree of rigidity persisted.

IV. MESENCEPHALIC REACTIONS IN DECEREBRATE RIGIDITY.

(*a*) *Median section through mesencephalon.* The importance of the superior cerebellar peduncles in the maintenance of decerebrate rigidity being fairly established, it was next endeavoured to ascertain the amount of decussation which occurs in the fibres of the peduncle concerned with rigidity. With this object in view, median sections of the midbrain from the region of the anterior colliculi caudad to the anterior limit of the pons were made. The cuts were followed by an immediate, initial accentuation of the rigidity and a later marked diminution, so that in the course of a few moments the degree of rigidity remaining was very small. This slight amount of rigidity, however, persisted throughout the observation, indicating a partial rather than a complete decussation in the tegmentum of the mesencephalon. Of course, there are two possibilities here:—the median section severs not only efferent paths (as regards cerebellum) but also any decussating fibre bundles from the midbrain to the cord. In either case, it is sufficient to realize that the decussation is apparently incomplete.

(*b*) *Gross excitation and inhibition of the midbrain.* Early in this series of observations, it was noted that exposure of the transected mesencephalon after decerebration resulted in a marked diminution or a total loss of the existent rigidity. Packing the cranial chamber loosely with cotton wool suffices to prevent ordinarily any diminution of the rigidity from this source. The natural procedure was to ascertain whether accentuation of the rigidity could be obtained by stimulating the cut surface of the midbrain. A warm saline swab (*circ.* 45°) applied to the transected surface immediately causes an extreme accentuation of the extensor rigidity already present. In a decerebrate animal, which after a complete cerebellar ablation exhibits a flaccid flexor attitude, the application of a warm saline swab to the midbrain likewise causes the development of a marked degree of extensor rigidity. In these cases, the excitation may persist for a long period (in one case, over an hour), gradually diminishing as the time passes. Ordinarily, the artificial rigidity persists only for a few minutes. Conversely, the application of a cold swab (*circ.* 16°) will cause a diminution of the existing extensor rigidity, while the placing of a bit of ice on the exposed surface will cause a very marked diminution or a total loss of the rigidity. The alternation of an exciting and inhibiting substance can be effected many times in the same animal. Painting the midbrain with a 1 °/₀ solution of cocaine hydrochloride does not seem to be as efficacious in causing a diminution in the rigidity as do the cold agents.

(*c*) *Unipolar exploration of the midbrain.* In a considerable series of decerebrate animals, the transected surface of the midbrain was explored with the stigmatic electrode. Horsley and Lowenthal have already obtained reactions from various parts of the midbrain; in the main, my results are similar to theirs. Stimulation of the mesencephalic tegmentum on either side of the raphe results, with weak current (Berne coil, 100 to 250 units), in a contralateral accentuation of the extensor rigidity at the elbow. With a stronger current (400 units) the contralateral extensor increase is more marked but added to it is an ipsilateral relaxation at the elbow. With still stronger current (500–700 units) the ipsilateral reaction may become a strong flexor rigidity at the elbow.

The application of this stronger current to both sides of the raphe simultaneously with two stigmatic electrodes results in the development of an extreme degree of bilateral extensor rigidity. Apparently then, the reaction is one adapted for progression, but the extensor element overpowers the flexor movement. The area from which these reactions are obtainable is that occupied by the cephalic portion of the nucleus ruber.

Stimulation of all other areas (with the exception of a single inhibitory area to be described below) ordinarily caused no effect on the rigidity. The middle three-fifths of the crura, containing the pyramidal fibres, with the ordinary strength of current used, gave no reaction, although in some cases contralateral limb movements were obtained. The colliculi apparently had no influence on rigidity.

The reactions from the tegmentum (region of the red nucleus) were obtainable from animals in which no extensor rigidity existed (*i.e.* those in which the cerebellum also had been removed or those in which exposure of the midbrain had abolished the rigidity). The ordinary reaction, then, was the contralateral extension at the elbow with an ipsilateral flexion at elbow also (if the current were somewhat stronger). These contractions did not persist after the cessation of the excitations.

(*d*) *Extirpation of one-half of midbrain.* With the gradual development of the conception of a mesencephalic origin of the motor portion of the reflex concerned in decerebrate rigidity, it was believed that possibly the extirpation of one-half of the midbrain would affect the existent rigidity. Hence, in one animal, a median cut was made through the mesencephalon caudad to the inferior border of the inferior colliculi. Then one lateral portion, divided off by this median section, was removed. The rigidity which was augmented by the median section quickly diminished bilaterally to an enduring, very slight degree. A similar sectional removal, which did not extend caudad beyond the cephalic border of the inferior colliculus, occasioned a considerable diminution of the rigidity on the opposite side with practically no effect on the ipsilateral rigidity. Later, in this same animal, the portion of the midbrain lying ventral to the inferior colliculus on the same side as concerned in the first extirpation was removed; the rigidity was diminished to a very small degree on both sides.

(*e*) *Transection just caudal to the caudal border of the inferior colliculi.* In several cases, the brain stem has been transected just caudal to the caudal border of the inferior colliculi. The ventral border of such a section usually coincides with, or lies just cephalic to, the cephalic limit of the pontine nuclei. In all of these cases there has been a short accentuation of rigidity, followed very quickly by a total loss of all rigidity with the assumption of a flaccid flexor attitude. In some cases, the rigidity has remained for five minutes after the transection of the brain stem, but ordinarily it has entirely vanished within two minutes. In no case, however, has any rigidity been appreciable longer than five minutes after the time of transection of the brain stem. This disappearance of the rigidity after such a section delimits, with a degree of certainty, the source of the rigidities.

V. THE INHIBITORY PATHWAY.

The fact that decerebration invariably is followed by an extensor rigidity suggested that there might possibly be some specific inhibitory representation in the cerebral cortex. This idea was further supported by the marked inhibitory responses obtained by unipolar stimulation of the anterior part of the superior vermis. For it seemed more than likely that this area in the anterior vermis represented the cerebellar link in the pathway from cerebral cortex to midbrain. This cerebellar pathway was suggested by Sherrington[1], but no evidence was given in support of such a view.

To ascertain the existence of such a pathway caudalwards from the cerebral hemispheres, various areas in the cut surface of the midbrain were stimulated in a decerebrate animal, in which a fairly constant degree of extensor rigidity existed. The stimulation was effected by the unipolar method, using customarily a very weak current (Berne coil, 100 to 250 units). With this strength of current, the tegmental nuclei (nucleus ruber) gave a mild accentuation of the extensor rigidity existent in the forelegs. When the stigmatic electrode with this excitatory current was applied to the mesial fraction of the cerebral peduncles, it was found that the rigidity entirely disappeared in the ipsilateral limbs, and was diminished in the contralateral. Exploration of this cut surface of the mesencephalon failed to reveal any other area which possessed an inhibitory influence upon the decerebrate rigidity. The pyramidal tract with this strength of current ordinarily gave no response; the response, if any, was always of a contralateral variety.

The area from which this inhibition can be obtained is, in the cat, quite small, comprising only the mesial one-fifth or one-sixth of the crus. These crura are quite close to each other, so that it is quite impossible to stimulate one side without stimulating the other, unless special means of insulation be resorted to. The effect, however, of exciting this area is almost entirely ipsilateral, as is easily proved by stimulation of the area on one side after the introduction, in the ventral midline of the mesencephalon, of a non-conducting plate. Such excitation leads to an ipsilateral inhibition of the rigidity with no apparent contralateral effect. It is correct then to consider this effect as a homolateral action and not as chiefly homolateral but still bilateral.

---

[1] *Loc. cit.*

The area of the midbrain ordinarily stimulated in these observations, which were made and verified in numerous cats, is that exposed in the technique of decerebration. Dorsal in this surface are the cephalic poles of the superior colliculi, while from these the surface slopes ventrally with a slight anterior deflection. The dorsum sellæ lies just anterior to the ventral margin—a factor of interference in the elicitation of the inhibitory response due to frequent oozing from the circular sinus.

After confirmation of the invariability of the inhibitory response from stimulation of the mesial part of the crus, the opportunity was offered to ascertain the possibility of a cerebellar pathway for the fibres excited. In the cat mentioned in a previous section in which extirpation of the cerebellum had no effect on rigidity, the inhibitory pathway was stimulated before and after the cerebellar ablation. With the cerebellum intact, the inhibitory response from excitation of this pathway in the midbrain was prompt and in accord with the other observations. After the cerebellar ablation, with the extensor rigidity still persisting, stimulation of the mesencephalic area was without effect upon the rigidity. The path, then, must traverse the cerebellum before exercising its inhibitory effect.

To delimit still further the exact course pursued by these inhibitory influences, a non-conductor was inserted into the ventral midline (without invading tegmentum) of the mesencephalon of a decerebrate cat. Stimulation of the crus on each side of this insulating plate resulted in invariable ipsilateral inhibition of rigidity. The middle cerebellar peduncle on one side was then sectioned without affecting in the least the extensor rigidity. Unipolar excitation then had practically its full effect in causing the inhibition of the ipsilateral rigidity, but stimulation of the contralateral area in the crus was without effect. From this it must be concluded that the path in the mesial fraction of the cerebral crura must ascend to the cerebellum by way of the contralateral middle cerebellar peduncle.

The experiments quoted thus far show the existence of an inhibitory pathway in the cut surface of the caudal mesencephalon, but the possibility of a thalamic origin for the pathway required exclusion before the assumption of a cortical source of the inhibitory fibres could be made. To delimit further the source of the inhibitory bundles, the cerebral hemispheres alone were removed from a cat. An extensor rigidity of customary degree made its appearance in this animal within two minutes after the extirpation of the hemispheres. As the animal gradually recovered from narcosis, walking movements were observed, rhythmic and existing in definite intervals. During the execution of these movements, the extensor rigidity was abolished to reappear as soon as the movements ceased. Sensory stimulation of mild degree and of almost any character sufficed as an excitant for this progression. Slight chloroformisation resulted in the abolition of the progressive movements but in the persistence of the extensor rigidity. After allowing sufficient time to elapse between the removal of the cerebral hemispheres and the subsequent procedures, the various areas in the exposed cut surface of the cerebral axis were excited by unipolar electrode (Berne coil, 250 to 400 units). The pyramidal fibres in the middle portion of the internal capsule yielded with the stronger currents contralateral movements. Excitation of the thalamus gave ordinarily no visible movement but at times contralateral movements of progression resulted. At the mesial anterior portion of the internal capsule an area was made out, about 4 mm. from the midline, which gave invariably inhibition of the ipsilateral rigidity. This inhibitory area was quite small and was symmetrically placed on both sides of the midline. Its reactions were quite prompt if a dry surface for excitation could be obtained, but with a moist surface the inhibition occurred only after five to fifteen seconds, and it frequently had movements of the neck muscles associated with it. In this case, the possibility of the inhibitory pathway having a thalamic origin was completely excluded.

In this same animal, successive cuts were made down to the caudal portion of the midbrain. The inhibitory pathway was after every section identified in its position in the mesial anterior fraction of the internal capsule and in the mesial fraction of the crura cerebri. The partial rotation of the fibre bundles from the anterior to the mesial surface of the pyramids could be easily verified. All rigidity disappeared in this animal after total transection was made caudal to the inferior border of the inferior colliculi. After removal of the thalamic regions, cessation of all progressive movements occurred.

With the course of this cortical inhibitory pathway, in the so-called fronto-pontine bundle, fairly delimited, an attempt was made in several cats to ascertain a possible cerebellar localization of the fibres. Most likely of the areas was that in the anterior portion of the superior vermis (the area, stimulation of which gave inhibition of the decerebrate rigidity). Consequently, after verification of the inhibitory area in the mesial portion of the crus cerebri, the cortex of the anterior portion of the superior vermis was extirpated. Immediately after this ablation, the rigidity became very much accentuated. Stimulation, then, of the inhibitory area in the crus had very much less effect than did the same strength of stimulation applied before the cerebellar destruction. The inhibitory effect of the excitation of the midbrain still continued, showing that the essential link in the cerebellum was not completely destroyed. The marked diminution in this inhibitory effect is, however, probably of some significance.

In animals in which, after decerebration, the cerebellar cortex in the anterior portion of the superior vermis was extirpated, inhibition of the extensor rigidity could be obtained by unipolar excitation of the subcortex in this portion of the anterior vermis. The reactions here required no more strength of current than from the cortex itself.

VI. ABNORMAL REACTIONS.

Under this heading it is proposed to mention briefly the reactions in the small number of experiments which failed to coincide with those of the vast majority of cases. Foremost among these is the observation, quoted on p. 213, in which the decerebrate rigidity remained unabated after complete extirpation of the cerebellum. The inconstancy of the unilateral loss of extensor rigidity following extirpation of one-half of the cerebellum has also been mentioned.

In two animals a flexor rigidity of marked degree has been observed. Both of these cases showed the same sequence in the development of the abnormal type of rigidity. Decerebration in both cases was followed by the customary degree of extensor rigidity. Both, then, subsequently began to bleed into the middle cranial chamber, and with this hæmorrhage the animals showed a complete loss of all rigidity with the assumption of a flaccid flexor attitude. On cessation of the hæmorrhage, the animals both began to develop slowly a flexor rigidity which, in the course of a half hour, became extreme. This flexor rigidity persisted until the animals were sacrificed. At autopsy, both cases showed herniation of the anterior portion of the vermis into the middle cranial chamber below the border of the ostentorium. In the posterior chamber a subdural blood clot, lying upon the basilar plate of the occipital bone, 3 mm. in thickness, was found in both. This association of cerebellar herniation and basilar hæmorrhage with the flexor rigidity may be of importance, but the exact significance cannot now be given.

In one other unusual case, decerebration was followed by the regular degree of extensor rigidity. In the course of an hour, the rigidity gradually disappeared entirely. There was no evidence of hæmorrhage into the middle cranial chamber. Removal of the bone over the cerebellum showed an extensive blood clot lying beneath the whole cerebellar dura. The removal of the bone over this caused no changes in the muscular reactions of the animal, but as soon as the cerebellar dura was opened and the clot removed, extensor rigidity developed and persisted as in the regular decerebrate animal. In still another decerebrate cat, the extensor rigidity on one side entirely disappeared. Exploration of the cerebellum showed in this animal a subdural clot over the cortex on the same side as that from which the rigidity had disappeared. Opening of the dura with removal of the clot and relief of any pressure upon the cerebellum were followed by the resumption of the extensor rigidity on that side.

VII. SUMMARY AND EVIDENCE FOR EXISTENCE OF PROBABLE PATHWAY OF REFLEX.

Consideration of the observations recorded in the foregoing sections leads one inevitably to the conclusion that the real origin of decerebrate rigidity lies in the midbrain in the region of the colliculi. This is substantiated in many experiments in which the rigidity absolutely disappeared on complete section of the brain stem below the caudal border of the inferior colliculi. The observations on the accentuation of the extensor rigidity by stimulation of the midbrain by hot saline swabs, and the diminution of the rigidity following the application of paralyzing agents to this cut surface, all point to the midbrain as the seat of the rigidity. The extensor increase obtained contralaterally by stigmatic excitation of the tegmental midbrain, with also bilateral extensor accentuation on bilateral simultaneous excitation, argues most strongly for a tegmental origin of the extensor rigidities. And in this portion of the midbrain, lying ventral to the colliculi, the tegmental structure which apparently affords origin for the rigidities is the nucleus ruber.

The connections of the red nucleus have been well known since the work of **von Monakow**, and these results must be correlated with the anatomic relations of the nucleus if it is to be given any importance as the point of origin of decerebrate rigidity. It is realized that it is practically impossible to affirm that any one nucleus is solely responsible for a reaction, but in this work it is felt that the superior and inferior limits of the nuclear origin of these decerebrate rigidities are well defined, and that if we assume the red nucleus to be the one important motor centre in the region under consideration, our results may be correlated to that region and nucleus. The nucleus ruber, as **von Monakow** showed, gives rise to a descending path, the rubrospinal bundle, which crosses the midline soon after its origin. Its decussation, however, is not complete. The fibres run spinalwards in the lateral portion of the rhombencephalic formatio reticularis, and, in the spinal cord, find representation just ventral in the lateral pyramidal tract. The other chief connection of the nucleus ruber is with the cerebellum. From the nucleus dentatus, an efferent tract (as regards cerebellum) comprises a large portion of the superior cerebellar peduncle; in this peduncle it almost wholly decussates and ends in large measure about the cells of the nucleus ruber.

We may proceed to discuss briefly the real source of the rigidities. **Sherrington**[1] showed conclusively that the rigidity is dependent upon the afferent impulses from any limb, indeed from any muscle of the antigravity set. But how do these impulses ascend the cord? Surely not in the posterior fasciculi, for section of these has no effect on the rigidity. **Sherrington** also demonstrated that section of the ventrolateral bundle of the cord abolished the rigidity in the ipsilateral limb caudal to the section. That the direct cerebellar tract (**Flechsig**) does not carry the necessary impulses upwards is proved by the fact that in these observations section of the inferior cerebellar peduncles (corpora restiformia) was without effect upon the extensor rigidities. The ventro-lateral tract, however, as **Mott**[2] has shown, chiefly goes to the cerebellum by way of the superior cerebellar peduncles. Some of the fibres, however, run to tegmental structures in the midbrain. All of the results recorded above may be explained on the basis that the ascending impulses concerned with decerebrate rigidity proceed to the cerebellum in the ventro-lateral fasciculus (**Gowers**). For the extensor rigidity disappears completely after section of both superior cerebellar peduncles. It is to be noted, however, that in section of these peduncles the cerebello-rubric tract is also divided. That none of the tracts (excluding the inhibitory pathway) concerned in decerebrate rigidity pass to or from the cerebellum in the middle or inferior peduncles has been shown in the observations given above.

---

[1] **Sherrington**, *loc. cit.*

[2] **Mott.** *Brain*, XVIII. p. 1. 1895.

The almost complete decussation of the pathway in the superior cerebellar peduncles or of the rubro-spinal tract is shown by the nearly total loss of rigidity which followed median cuts through the midbrain. For with these cuts were severed the decussating fibres of the superior cerebellar peduncle and of the rubro-spinal tract. Further evidence of this incomplete decussation lies in the great loss of rigidity on one side which follows section of the ipsilateral superior cerebellar peduncle. Two explanations of this ipsilateral loss are possible: section of the ventro-lateral fasciculus may have caused this loss of rigidity on the same side, or the dentato-rubric fibres were sectioned, cutting off necessary impulses to the contralateral red nucleus, from which the majority of the rubro-spinal fibres immediately decussate, with, hence, an ipsilateral diminution of rigidity. Further evidence of this partial decussation in the midbrain is given in the observations made after removal of one-half of the midbrain with almost total loss of rigidity on both sides.

Our evidence, then, points to a definite cerebellar link in the reflex chain necessary for the development and maintenance of decerebrate rigidity. Certainly the observations recorded above show that the simple exposure of the cerebellar cortex before the removal of the cerebral hemispheres is sufficient to prevent the development of any extensor rigidity. Likewise, in an acute experiment, the primary removal of the whole cerebellum prevents the development of these enduring contractions. And, after the development of decerebrate rigidity, subsequent complete cerebellar ablation in practically all acute experiments leads to its total loss. In addition, the application of paralyzing agents to the cerebellar cortex diminishes while the stimulation of the cortex by hot swabs increases enormously the rigidities. We have evidence, then, of a probable essential cerebellar link in the causation of these decerebrate rigidities.

With regard to the one observation in which loss of rigidity did not follow the cerebellar ablation, it may perhaps be assumed that in this animal the fibres in the ventro-lateral fasciculus, which ran directly to the midbrain, were sufficient to account for the persistence of the rigidity. Similarly, in the animal in which the whole cerebellum had been removed some weeks previously, the persistence of the rigidity for two hours and a half, though of slight degree, may have been due to this direct mesencephalic termination of the fibres in this ventro-lateral fasciculus.

The question of the cerebellar cortex playing an essential *rôle* in decerebrate rigidity naturally calls for solution in view of the work of Edinger[1] and of Horsley and Clarke[2] on the intimate structure of the cortex. The simple exposure of the cortex was sufficient in these observations to prevent the development of decerebrate rigidity, provided the cerebellum be exposed before decerebration be done. The effect of paralyzing and stimulating agents applied to the cerebellar cortex also must be taken as an argument for the essential influence of the cerebellar cortex. Destruction of the cortex was attempted in one experiment, but it had but little effect on the existent rigidity. However, the absolute impossibility of destroying more than a small part of the cerebellar cortex without injury to subcortical structures is to be assumed from consideration of the anatomical relations of the parts of the cerebellum. The evidence that presents, although not wholly conclusive, surely argues for representation of the rigidity reflex in the cerebellar cortex.

In the recorded observations, it may be noted that some attempts were made to obtain evidence of cerebellar localization for the rigidities of the various parts of the body. It was found that extirpation of one-half of the cerebellum in most cases caused the total loss of all rigidity in the fore and hind legs on the same side. But in about one-fourth of the cases no effect on rigidity was obtained by this partial ablation. If the half of the cerebellum be removed before decerebration, the extensor rigidity developed on both sides with but little retardation on the side from which the cerebellar lobe was removed. The removal of one lateral lobe of the cerebellum, after decerebrate rigidity had developed, caused diminution of the rigidity in the ipsilateral legs, but had no effect on the rigidity in the tail. Removal of the whole vermis region did not interfere with the development of bilateral extensor rigidity, but the tail also showed an extensor tendency. Consideration of these observations and other phenomena observed led to the conclusion that, although there was some evidence for cerebellar localization in the rigidity of the extremities, it was quite imperfect, and that in most cases, it was better to consider the cerebellum as a unit in the pathway. Undoubtedly, however, the localization of the inhibitory pathway in the cerebellar cortex is very definite, and any interference with it is followed by definite changes in the rigidity.

[1] Edinger. *Loc. cit.*

[2] Horsley and Clarke. *Loc. cit.*

Considering that the extensor contractions in decerebrate rigidity were the reactions of the animal against gravity, it seemed more than likely that the semi-circular canals might be implicated. **Sherrington** had already described section of the eighth nerve in a monkey as being without effect upon the subsequent development of decerebrate rigidity. In investigation of this matter, two cats were used. In both, the usual decerebration was done with the development of the extensor rigidity. Then from above, after removing the calvarium, the internal ears were completely destroyed. The rigidity in both animals remained absolutely unaffected by the procedure, excluding the vestibular apparatus from any part of the phenomenon. Further support of this view that the semi-circular canals are not involved is given by the fact that section of the inferior cerebellar peduncles has no effect on the rigidity. For the close relation of vestibule to cerebellum through this corpus restiforme, suggests that were these canals involved, the restiform body would undoubtedly carry a portion of the necessary tract.

The inhibitory pathway apparently arises from the cerebral cortex, runs caudad in the anterior mesial portion of the internal capsule, continues downward in the mesial fraction of the crus cerebri and probably ends in the pontine nuclei. The second relay takes it from the pons to the cerebellum by way of the contralateral middle cerebellar peduncle, whence it is distributed, in part at least, to the cortex of the anterior portion of the superior vermis. **Sherrington's** results upon the development of an ipsilateral rigidity on removal of one cerebral hemisphere are in accord with the ipsilateral inhibition of rigidity obtained by stimulating this tract anywhere in its mesencephalic course. There is no evidence of the further course of this tract after it reaches the cerebellar cortex. Whether it exercises its inhibitory effect upon the neurones in the basilar cerebellar nuclei or upon the mesencephalic nuclei cannot be told from these observations.

With the realization, then, that there exists an inhibitory as well as an excitatory influence on rigidity in the cerebellum, it does not appear extraordinary that in the acute experiments, the cerebellar extirpations should at times be followed but slowly by the loss of extensor rigidity. For necessarily, the extirpation must be accompanied by a stimulation of all the cerebellar pathways, and in this excitation the inhibitory and excitatory may be alike stimulated. So that in these observations, in addition to the usual difficulty of distinguishing between the stimulation of extirpation and the subsequent paralysis, there is also the greater difficulty of distinguishing between stimulation of inhibitory fibres and paralysis of excitatory, and *vice versa*.

CONCLUSIONS.

From the above observations the following conclusions may be drawn:

(1) The main reflex centre and, in that sense, the source of decerebrate rigidity lies in the midbrain and is probably the nucleus ruber.

(2) The cerebellum forms a very important, if not absolutely essential, pathway for the afferent impulses from the limbs and trunk, concerned in decerebrate rigidity.

(3) The cerebellum forms an essential link in the inhibitory pathway from the cerebral cortex.

(4) The localizations of the cerebellum, as far as can be ascertained from study of the rigidities in the various extremities, are not sharply circumscribed or very minutely differentiated.

IS POLISHED RICE PLUS VITAMINE A COMPLETE FOOD?

By CASIMIR FUNK.

(From the Department of Chemical Physiology, Cancer Hospital Research Institute, Brompton, London, S.W.)

In previous papers on this subject (1) I have described in detail the methods of preparation of the vitamine-fraction from yeast and other foodstuffs and also the curative action of this substance on beri-beri pigeons. Since then several authors, whose work is fully discussed elsewhere (2), have expressed the view that the vitamine-fraction isolated in this way, although possessing very marked curative effect, is unable to sustain life and maintain the weight of birds fed on polished rice.

Since my last publications on the chemistry of the vitamine-fraction I have repeated the fractionation of yeast three times, following exactly the same procedure, and have succeeded in each case in isolating a crystalline vitamine-fraction, melting at 210–213° with the chemical characters described. Having thus prepared a few grams of this substance I thought it advisable to investigate fully its curative and protective value. Not less than 22 pigeons were cured with this preparation, the dose varying from 2–4 mgr. administered intramuscularly. The cure was effected usually in less than three hours and the effect lasted as a rule for 3–7 days, the pigeons being kept exclusively on polished rice. After this time the symptoms appeared again, in five cases however I succeeded by an administration of a second dose in curing the disease again, but the effect lasted only for two days. In four cases it was attempted to keep pigeons on polished rice by injecting one mgr. of the vitamine-fraction every day, but it was impossible to keep the birds alive for more than nine days. If however after the injection of the curative dose or even two days after the cure, normal food (maize) was given, the birds remained healthy. We have at present in our institute three pairs of pigeons, which were cured by injection of vitamine nine months ago, and which have subsequently bred and produced perfectly healthy, normal young birds.

Besides its action on beri-beri, the effect of injections of vitamine-fraction from yeast on the power of stimulating the growth of young chicks, which had been arrested by feeding exclusively on unpolished (red) rice, was investigated, as reported elsewhere (3), but not the slightest effect of the injections was observed.

These experiments show that although by the methods of isolation of the vitamine-fraction, described in my previous papers, one can extract a substance from yeast, rice-polishings etc., which cures beri-beri in pigeons, and as Vedder and Williams (4) have shown also in man and fowl, this substance loses the power possessed by the material from which it was extracted (yeast, rice-polishings) of maintaining life and weight of the animals in experiment.

It also was observed that the vitamine-fraction could be kept in a crystalline state for over eight months without losing a trace of its curative power.

Recently I have obtained the vitamine-fraction from yeast by a method which will be described later on, the action of which is so complete that I thought it worth while to publish the experiments. For the sake of economising the material only six pigeons were used. The birds were kept exclusively on polished rice throughout the experiment. Four of these pigeons were cured by a single injection of the new vitamine-fraction, they recovered in time varying from one to three hours, showing appetite immediately on recovery, increased in weight during the first days after the cure and maintained the gained weight for about a week. After this time they slowly lost weight again and developed beri-beri after 12, 14, 14 and 15 days respectively. The two other birds which recovered after one injection, fed themselves on polished rice, the dose of vitamine being repeated every four days. There was no evidence of beri-beri symptoms. The two pigeons lived for 29 and 32 days respectively and eventually died of sepsis, as shown by the post mortem examination, due to the curative solution being kept for over a month. The sterilisation was not possible on account of the destruction of vitamine by heat. The post mortem examination also showed, that there was no disappearance of thymus, which always takes place as shown in collaboration with Dr Mackenzie Douglas (5) in beri-beri pigeons.

It must be especially emphasized that the new vitamine-fraction was also entirely devoid of phosphorus and possessed at the same time the anti-neuritic, appetising and weight-keeping properties. This in my opinion proves that polished rice plus vitamine constitutes a complete food. These experiments show at the same time that contrary to the opinion expressed by several authors there is no necessity, in any case at the present moment, to assume the existence of several vitamines, one for curing the nervous symptoms and another one for the maintenance of the body weight.

The fact that even this complete life-sustaining action is brought about without the help of lipoids or other substances containing phosphorus shows that the physiological importance of lipoids in this regard must not be attributed to lipoids but to vitamines which in the crude state are soluble in lipoid-solvents.

Experimental.

Out of the four pigeons used for the single dose curing experiments, one which was fed on the so-called synthetical diet (caseinogen, starch, fat, sugar and mixture of salts), according to the experiments which were described elsewhere (8), developed typical beri-beri in 29 days. This animal was injected with 2 mgr. of the vitamine-fraction and completely recovered three hours afterwards. The weight of the bird which immediately after recovery ate polished rice freely, was as follows:

24/2/14	...	...	200 gms.
25/2/14	...	...	220 „
1/3/14	...	...	210 „
9/3/14	...	...	180 „

On Mar. 10, that is to say 14 days after recovery the beri-beri symptoms reappeared, the bird died on the 13th.

The other three birds in this experiment developed beri-beri on polished rice. They were treated with a dose containing two mgr. of the vitamine-fraction injected intramuscularly and recovered between 2–3 hours after the injection. Two days after the recovery they gained between 10–20 grm. in weight, a gain which was maintained throughout the first week. These three pigeons developed beri-beri after 12, 14 and 15 days respectively.

Two other pigeons which developed beri-beri on polished rice after 20 and 22 days respectively were injected with 2 mgr. of the solution of the crystalline vitamine-fraction. One bird was completely cured after three hours, the other after two hours. Immediately after the cure they began to pick up the rice and fed themselves throughout the experiment. Every few days the injections were repeated and the birds were kept in permanent health one for 29 the other for 32 days. As the weight of the birds were very near each other, both animals were weighed together and the average weight recorded. The table below shows the time of the injections and the weights.

Day	Injections	Weights
1	2 mgr. each	220 gms.
4	—	240 „
6	2 mgr.	245 „
7	—	260 „
8	2 mgr.	—
10	—	255 „
11	1 mgr.	—
18	1 mgr.	250 gms.
17	1 mgr.	250 „
19	1 mgr.	—
21	—	240 „
22	1 mgr.	—
25	—	242 „
29	1 mgr.	—

The two birds died without a trace of beri-beri symptoms and the post mortem examination has shown that in both cases the cause of death was sepsis, pyaemic abscesses being found in the liver. The sepsis was due to the fact that the solution of the vitamine which was kept in the cold room for over a month could not be sterilised. In both cases the thymus gland presented the appearance seen in healthy birds.

CONCLUSIONS.

1. The experiments show conclusively that polished rice and vitamine constitute a complete food.

2. There is no justification at the present moment to accept the necessity of two different vitamines, one for curing the nervous symptoms and one for maintenance of body weight.

3. The vitamine-fraction used in the above experiments being entirely free from phosphorus we are justified in saying that the physiological importance ascribed in late years to lipoids and substances soluble in lipoid solvents as regards their bearing on deficiency diseases must not be attributed to lipoids but to vitamines which are accidentally extracted in the same process.

REFERENCES.

(1) Casimir Funk. Brit. Med. J. 19th April, 1913; This Journ. XLVI. p. 173. 1913; Ibid. XLIII. p. 395. 1911.

(2) Casimir Funk. Die Vitamine. Bergmann, Wiesbaden, 1914.

(3) Casimir Funk. The Lancet, Jan. 10th, 1914.

(4) Vedder and Williams. The Philippine J. of Sc. VIII. Sect. B. Tropical Medicine, p. 175. 1913.

(5) Casimir Funk and Mackenzie Douglas. This Journ. XLVII. p. 475. 1914.

(6) Casimir Funk. Ztschr. f. physiol. Chem. LXXXIX. p. 373. 1914; LXXXIX. p. 378. 1914.

THE FORMATION OF URINE IN THE FROG. BY F. A. BAINBRIDGE, J. A. MENZIES AND S. H. COLLINS.

(From the Physiological Laboratory, University of Durham College of Medicine.)

IN a previous communication (2) we have shown that, when the frog's kidneys are perfused simultaneously through the aorta and renal portal veins with oxygenated Ringer's solution, the urine obtained is hypotonic to the perfusing fluid and is derived solely from the glomeruli, since the tubules form no urine. The perfusion was made at pressures approximating to those normally observed in frogs with intact circulation, namely 20 to 24 cm. of water for the arterial perfusion and 10 to 12 cm. for the venous perfusion. Evidence was also brought forward that the glomeruli form by filtration urine isotonic with the perfusing fluid, and that, during the passage of the glomerular filtrate down the tubules, the latter usually absorb sodium chloride.

The present paper deals first with some further experiments in support of this view, and secondly with the alleged capacity of the tubules to secrete urine on a venous perfusion only under a high pressure (25–40 cm. of water).

I. *Absorption by the tubules.*

The methods adopted and the solutions used were similiar to those previously described. The kidneys were first perfused through the aorta and renal portal veins with oxygenated Ringer's solution. Subsequently a weak solution of corrosive sublimate was perfused through the renal portal veins for a short time in order to poison the tubules, the arterial perfusion with Ringer's solution being continued. Simultaneous arterial and venous perfusion with Ringer's solution was then resumed. At the end of the experiment the kidneys were perfused through the renal portal veins with weak ammonium sulphide in Ringer's solution, hardened in alcohol and examined histologically. The molecular concentration of the Ringer's solution and of the urine was determined by means of the refractometer.

Histological demonstration of the absence of mercuric sulphide from the glomeruli is obviously not easy, although its presence in the tubules offers a contrast which is often very helpful. So far as we can judge, however, the glomeruli were in most cases free from mercuric sulphide, but in some kidneys mercury appeared to be definitely present in the glomerular capillaries and it seemed probable that the sublimate had reached them by diffusion (Fig. 1). The latter experiments were disregarded. The best results were obtained by perfusing corrosive sublimate for 2–3 minutes under a very low pressure. Under these circumstances the ventral part of the kidney is sometimes free from mercuric sulphide and the glomeruli entirely escape, although the convoluted tubules contain sulphide. When the perfusion of the kidney with corrosive sublimate is more complete, the possibility of its diffusion into the glomeruli is clearly increased, although it does not invariably occur. It does not seem probable that the mercuric salt reaches the glomeruli by diffusion and is subsequently washed away by the arterial perfusion, since it forms an insoluble compound with protein. It is not washed out of the tubules, which are equally exposed to the perfusing fluid.

Numerous experiments have been made by the method just described. They confirm our previous results as regards both the composition of the urine and the histological appearance of the kidneys. The latter is illustrated in Fig. 1. These results, therefore, support the view that the glomeruli form by filtration a urine isotonic with the perfusing fluid, and that, during the passage of this filtrate down the tubules, sodium chloride is absorbed by them. It may be noted that occasionally, even in the normal kidney, the urine may be isotonic or almost isotonic with the perfusing fluid, so that the absorption of sodium chloride is not invariably taking place in the frog.

TABLE I. *The effect of poisoning the tubules with Ringer's solution containing* 1/10,000 *corrosive sublimate.*

	Concentration of urine			
Exp.	Normal kidney	Poisoned kidney	Conc. perf. fluid	
1	0·41 %	0·54 %	0·56 %	Glomeruli free from HgS. HgS visible in tubules.
2	0·43	0·57	0·54	
3	0·55	0·65	0·72	

Some further experiments were made on the action of caffein on the tubules. It was found that the perfusion for five minutes of

0·5 to 1 % caffein sodium benzoate through the renal portal veins usually caused the urine to become isotonic with the perfusing fluid, but only for a comparatively short time; within half an hour or so the urine was again hypotonic to the perfusing fluid. The effects of weaker solutions of caffein were inconstant. Throughout the whole of each experiment a simultaneous arterial perfusion of oxygenated Ringer's solution was maintained. The results of these experiments are inconclusive and unsatisfactory, but, so far as they go, they suggest that caffein may inhibit absorption by the tubules for a short time.

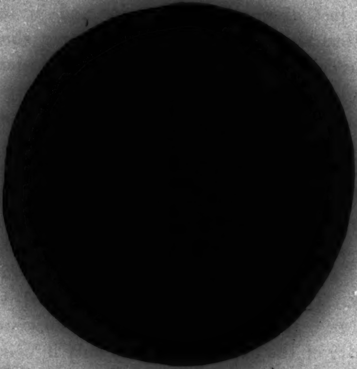


Fig. 1. Microphotographs showing on the left glomeruli containing mercuric sulphide, on the right glomeruli free from sulphide. In each case the epithelium of the tubules contains HgS.

II. *Venous perfusion experiments.*

In our former experiments we found that, when the kidneys were perfused with oxygenated Ringer's solution through the renal portal veins alone (venous perfusion) under a pressure of 10–12 cm. of water, no urine was obtained except in very rare cases. Miss Cullis (4) has shown, however, that on a venous perfusion alone urine is readily obtained if urea, phloridzin, or dextrose be added to the perfusing fluid, the perfusion pressure being from 25 to 40 cm. of water. She found that when the perfusing fluid was simple Ringer's solution little or no urine was formed even under high pressure. It appeared to us desirable first to determine by the aid of the refractometer the composition of the urine obtained under these circumstances, and secondly to ascertain whether any relation existed between the perfusion pressure and the amount of urine obtained.

(*a*) *Methods.* In the first instance the following method was used, the experiments being made on fully pithed male frogs. A cannula was placed in the left aortic arch (the right arch being tied off) and an arterial perfusion was started as quickly as possible through the aorta at a pressure of 24 cm. The cœliaco-mesenteric artery was tied, the hind legs were tied off as high up as possible, and the testes and fat bodies were removed by the cautery. Cannulæ were then placed in the inferior end of the divided anterior abdominal vein, in the vena cava just above the renal veins and in the ureters. The kidneys were then simultaneously perfused for a short time through the aorta and renal portal veins, the venous perfusion pressure being 10–12 cm. of water; the perfusing fluid was oxygenated Ringer's solution. As soon as a sample of urine had been obtained the arterial perfusion was shut off, and the venous perfusion was continued, sometimes at the same pressure as before, and sometimes at a pressure of 28–30 cm. At the end of each experiment the kidneys were perfused through the renal portal veins for 5–10 minutes with Berlin blue solution under a pressure of 28–30 cm. of water. The kidneys were fixed in alcohol and serial sections were cut and examined.

(*b*) *Results.* The amount of urine obtained varied with the perfusion pressure. When the latter was low, no urine was formed in the great majority of the experiments, and only in one or two instances were small traces obtained. When the pressure was high, urine was almost invariably formed, the amount varying from one-half to one-tenth of that yielded in the same time on a combined arterial and venous perfusion. When examined by the refractometer the urine was hypotonic to the perfusing fluid.

Exp. 4. Pithed male frog. Cannulæ in left aortic arch, anterior abdominal vein, vena cava and ureters. Hind legs tied off, cœliaco-mesenteric artery and right aortic arch ligatured, testes removed. The perfusing fluid was oxygenated Ringer's solution.

Time	Arterial pressure	Venous pressure	Perfusion rate	Urine
10.45–11.5	24 cm.	12 cm.	34 c.c.	R. 0·15 c.c. L. 0·1 ,,
11.5–11.25	24	12	28	R. 0·2 ,, L. 0·15 ,,
11.25 arterial perfusion shut off.				
11.25–11.45	0	29	52	R. 0·05 ,, L. 0·05 ,,
11.45–12.5	0	29	50	R. 0·15 ,, L. 0·05 ,,

In other experiments in which 0·2 to 0·4 % urea was added to the Ringer's solution the results were similar to those just described, and in these also the urine was hypotonic to the perfusing fluid. We found however that, as noted by Miss Cullis, the addition of urea sometimes led to a more rapid rate of perfusion than when plain Ringer's solution was used.

Exp. 6. Pithed male frog. Dissection as in Exp. 4. Perfusing fluid, hypotonic Ringer's solution with 0·4 % urea added.

Time	Arterial pressure	Venous pressure	Perfusion rate	Urine
10.40–11	28 cm.	13 cm.	33 c.c.	R. 0·05 c.c. L. 0·075 „
11–11.20	28	13	31	R. 0·12 „ L. 0·15 „
Arterial perfusion shut off at 11.20.				
11.20–11.40	0	13	19	Nil.
11.40–12	0	29	62	R. 0·075 c.c.
12–12.20	0	29	61	L. 0·075 „

The concentration of the urine as measured by the refractometer in these experiments is shown in Table II.

TABLE II. *Molecular concentration of perfusing fluid and urine calculated as percentage of NaCl.*

		Molecular concentration of	
Exp.	Perfusing fluid	Perfusing fluid	Urine
6	Ringer + 0·4 % urea	0·85 %	0·68 %
7	„ „	0·87	(*a*) 0·55 (*b*) 0·65
8	„ „	0·88	0·59
9	Ringer's solution	0·50	(*a*) 0·29 (*b*) 0·41
10	Ringer + 0·2 % urea	0·65	(*a*) 0·46 (*b*) 0·60

The figures lettered (*a*) and (*b*) in some of these experiments refer to successive samples of urine. All the samples of urine referred to in this table were obtained on a venous perfusion only.

In some of the experiments described in Table II. it will be observed that the perfusing fluid used was by no means a pure solution of sodium chloride. The percentage of sodium chloride (with potassium and calcium chloride) was approximately 0·5 % and that of urea was 0·4 %. In our former communication we pointed out that the refractometer gave readings proportionate to the true molecular concentration because the fluids employed were almost pure solutions of one substance. It is necessary now to enquire how nearly approximate the refractometer readings are in the case under discussion. Using solutions of 1 °/₀ or less the refractometer readings for urea are 82 °/₀ of those with sodium chloride of the same molecular concentration. A solution of 0·5 °/₀ NaCl + 0·4 °/₀ urea will therefore read on the refractometer as 0·82 °/₀ NaCl.

The dilution of the urine as compared with the perfusing fluid might be produced by the absorption either of sodium chloride or of urea or both; it might also result from the absorption of some sodium chloride and the simultaneous secretion of a small amount of urea. The results do show, however, that the urine formed differs from that normally formed by the secreting kidney, which has a higher molecular concentration than that of the blood.

In every experiment in which a measurable quantity of urine was obtained a variable number of glomeruli were found to contain Berlin blue when the kidneys were examined histologically, whereas when no urine was obtained the glomeruli were free from blue. When only minute traces of urine were formed, blue was present in a few glomeruli in some experiments but not in others. It may be pointed out, however, that the Berlin blue solution is distinctly more viscid than saline solution and passes along narrow channels much less readily. The relationship between the formation of urine during the experiment and the histological appearance of the kidneys is illustrated by the following figures. In seven experiments urine was formed in sufficient amount (0·1 c.c. or more) to yield a sample for examination by the refractometer, and in all of them a certain number of glomeruli contained Berlin blue. In three experiments no urine at all was obtained and blue could not be detected in any of the glomeruli; a large number of serial sections was examined. In two experiments traces of urine were obtained; in one of these a few glomeruli contained blue, in the other no injected glomeruli were found. The observation that, broadly speaking, whenever urine was formed some glomeruli were injected with Berlin blue seems to offer fair ground for the conclusion not only that these glomeruli were in circulation during the experiment, but also that they were capable of forming and actually did form urine. The assumption that the urine is formed solely or even partially by the tubules and is a true secretion does not seem justifiable for two reasons. In the first place, the fact that in experiments in which no urine was formed no glomeruli could be injected renders it probable that under the conditions of our experiments urine is formed exclusively by the glomeruli.

Secondly, the character of the urine obtained in these cases is not in favour of its being a secretory product.

(*c*) As a check upon these experiments the attempt was made to put the glomeruli completely and permanently out of circulation at the beginning of the experiment by the following method. Immediately after the frog was pithed the anterior abdominal vein was ligatured and divided. A cannula was placed in its inferior end, but the venous perfusion was not started. The hind legs were tied off, the aorta was tied off at its bifurcation into the iliac arteries and all the branches leaving the aorta except those going to the kidneys were as far as possible obliterated by the cautery. The testes and fat bodies were removed with the aid of the cautery, and a ligature was placed round both aortic arches just above their junction to form the main aorta. These proceedings took only a few minutes, during which the circulation through the kidney was not disturbed. The ligature round the aortic arches was then tied, the heart was excised, and the venous perfusion was immediately started. A ligature was tied round the rectum, and the intestines were removed with the aid of the cautery. Cannulæ were then placed in the vena cava and the ureters. This procedure left the aorta, renal arteries and glomeruli full of blood without interfering with venous perfusion through the intertubular vessels. The venous perfusion was carried on for an hour or more under a high pressure, and at the end of the experiment the kidneys were perfused at the same pressure for ten minutes through the renal portal veins with Berlin blue solution. They were then hardened in alcohol and examined histologically. Five experiments of this kind were carried out. In three no urine was obtained and no injected glomeruli were found. In the other two experiments traces of urine were formed and a few glomeruli contained blue. The large majority of the glomeruli were full of blood though some were found here and there washed partially free of blood. Although the method does not always result in the glomeruli being put completely out of circulation, yet the experiments in which no urine was formed and no glomeruli were injected support the view that, when urine is formed on a venous perfusion alone under a high pressure, it is derived exclusively from the glomeruli.

(*d*) Our observation that in many experiments the glomeruli could be injected through the renal portal veins was rather unexpected, and we have carried out a large number of experiments in order to ascertain the actual route by which the Berlin blue (and perfusing fluid) reach the glomeruli. There is no doubt that even on a venous perfusion alone, and after removal of the testes and fat bodies, the perfusing fluid can reach the arterial system. In a number of experiments the kidneys were first perfused simultaneously by the aorta and the venous system, and then the arterial cannula was disconnected from the perfusion bottle, while the venous perfusion was continued under a high pressure: in some of these a very slow flow of fluid along the arterial cannula took place, and the Berlin blue injected at the end of the experiment often entered the cannula and passed some little way along it. Subsequent examination of the aorta itself showed that it contained Berlin blue. At first it seemed probable that, under the high perfusion pressure, anastomoses were opened up between the renal portal veins and the arterial system. Such paths might be offered by the small veins from the abdominal muscles, the bladder and the rectum, which open into the inferior part of the anterior abdominal vein. There is in fact a fairly free venous anastomosis from the anterior abdominal vein through the rectum along the alimentary canal as far as the liver; this can be excluded by tying off the bladder and rectum. If, during a venous perfusion experiment, the bladder and rectum being excluded in this way, the renal portal veins be ligatured close to the kidneys, the result is an absolute and immediate cessation of the flow of urine and of the escape of the perfusing fluid from the vena cava. Another possible route is by the lumbar veins, four or five on each side, which open into the renal portal vein or its branches on the dorsal surface of the kidney. This channel is easily stopped by division of these veins, preferably by means of the galvano-cautery. The third possible indirect path from renal portal veins to glomeruli is that through the testes and fat bodies. This is blocked by removal of these structures. In order to preclude any possibility of the perfusing fluid reaching the glomeruli from the renal portal veins by any circuitous channel, we have performed a number of experiments in which, after adopting all the above-mentioned precautions, we have excised the aorta from above the junction of the arches to below the bifurcation. In six such experiments urine was still formed; in two the amount was sufficient for examination by the refractometer. In every experiment a certain number of glomeruli contained Berlin blue, and in some instances the injected glomeruli were in the middle third of the kidney, which must have been absolutely severed from any connection with the aorta.

Exp. 11. Pithed male frog. Initial dissection as in Exp. 4. Testes and fat bodies removed by the cautery, bladder and rectum ligatured, lumbo-renal veins divided by cautery. Perfusing fluid oxygenated Ringer's solution. After a preliminary arterial and venous perfusion in which urine was obtained, the arterial perfusion was shut off and the aorta completely excised.

Time	Venous pressure	Perfusion rate	Urine
3.25–3.45	29 cm.	35 c.c.	R. 0·04 c.c. L. 0·04 c.c.
3.45–4.5	29	17	Small amount.
4.5–4.15	Berlin blue perfused at 29 cm. pressure.		

Histologically Berlin blue present in some glomeruli which lie in the middle third of the kidney.

In view of these experiments it is difficult to understand how the Berlin blue and the perfusing fluid can reach the glomeruli from the arterial side. The evidence suggests that under the conditions of our experiments the perfusing fluid forces its way along the efferent vessels of the glomeruli into the capillaries and so into the afferent vessels and renal arteries. These conditions are (1) a very high venous pressure, and (2) that the glomerular vessels are already free from blood and full of saline solution when the high pressure venous perfusion is started. There does not seem to be any histological difficulty in accepting this view since (Fig. 2) the efferent vessel is not notably smaller than the afferent and it opens directly into the network of intertubular vessels. The resistance offered by the efferent vessels will

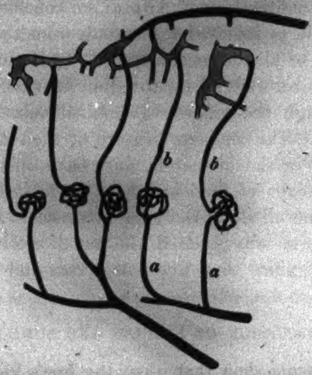


Fig. 2. Drawing of blood-vessels in a frog's kidney. The renal arteries were injected with carmine-gelatin, the renal portal veins with Berlin blue gelatin. The efferent vessels from the glomeruli, and a small branch of the renal portal vein, are shown opening into the commencement of the intertubular vessels.

naturally be much greater than that of the wide intertubular capillaries and only a small and incomplete perfusion of the glomeruli can be expected. Such a reversal of the normal direction of the flow of fluid through the glomeruli is obviously impossible in frogs with an intact circulation, or even with venous perfusion at pressures approximating to those observed in the normal animal. Nor does this reversal take place as a rule when the aorta is tied at both ends (see section (c)), and all its branches, except those going to the kidney, are ligatured, so that the glomeruli and their efferent and afferent vessels are full of stagnant blood.

This suggestion as to the route by which Berlin blue and the perfusing fluid reach the glomeruli does not appear, therefore, to be at variance with the observations of Nussbaum(8) and of Beddard(2), nor is it in any way confirmatory of Adami's(1) conclusions. Nussbaum found it impossible to inject the glomeruli from the venous side after complete ligature of all the renal arteries. In Beddard's experiments also the renal arteries were all ligatured an hour before the injection of Berlin blue through the anterior abdominal vein and the glomeruli were full of blood. The complete ligature of the renal arteries and the presence of blood in the glomeruli must have offered an insuperable obstacle to the escape of fluid and have made it impossible to force fluid into the glomeruli along the efferent vessels. In our experiments in which the aorta was excised the renal arteries were at least potentially patent, since they were cut across and not cauterised.

DISCUSSION OF RESULTS AND SUMMARY.

These experiments show that when the frog's kidneys are perfused through the renal portal veins alone under a high pressure (1) the kidneys usually form urine whether the perfusing fluid is oxygenated Ringer's fluid or whether urea or other diuretics are also present; (2) the urine is hypotonic to the perfusing fluid; (3) whenever urine is formed the perfusing fluid reaches and traverses the glomeruli. It is obvious that since the Berlin blue enters the glomeruli, the Ringer's solution which is perfused for a much longer period under the same pressure must take the same course. Evidence has also been given that under the particular conditions of these experiments the perfusing fluid in all probability makes its way into the glomeruli along the efferent vessels and not through the renal arteries. If these results are valid it is clear that a venous perfusion under a high pressure is rarely confined to the intertubular vessels, but that it generally includes the glomeruli and therefore differs but little from an ordinary arterial perfusion of the kidney, except that the glomerular pressure is higher in the latter and the perfusion of the glomeruli is more complete. It is only in experiments in which the glomeruli do not contain Berlin blue that one is justified in assuming that the perfusion is limited to the intertubular vessels; and in such experiments no recognisable amount of urine is formed by the kidneys. Moreover, the fact that on a venous perfusion only a high pressure is necessary for the formation of urine appears in itself to be an argument against the urine being a true secretion; if the tubules can form urine in these perfusion experiments there seems no reason why they should not do so under a low perfusion pressure, since even then the supply of oxygen to the tubules is very considerable.

The evidence seems to us, therefore, opposed to the view that under the conditions of these experiments the urine formed is secreted by the tubules, although the possibility that the tubules may secrete very minute traces of urine cannot be absolutely excluded. We consider that the urine is derived entirely from the glomeruli, and that as it passes down the tubules sodium chloride is absorbed, and the urine collected from the ureters is therefore hypotonic to the perfusing fluid. The amount of urine formed is usually small because the perfusion of the glomeruli (judged by the number containing Berlin blue) is incomplete as compared with that taking place on an ordinary arterial perfusion.

The expenses of this research have been defrayed by a grant from the Royal Society.

REFERENCES.

(1) **Adami.** This Journal, VI. p. 382. 1885.
(2) **Bainbridge, Collins and Menzies.** Proc. Roy. Soc. B, LXXXVI. 1913.
(3) **Beddard.** This Journal, XXVIII. p. 20. 1902.
(4) **Cullis.** Ibid. XXXIV. p. 250. 1906.
(5) **Nussbaum.** Anat. Anzeiger, I. p. 67. 1886.

THE ABSORPTION AND DISSOCIATION OF CARBON DIOXIDE BY HUMAN BLOOD. BY JOHANNE CHRISTIANSEN, C. G. DOUGLAS AND J. S. HALDANE.

(From the Physiological Laboratory, Oxford, and the Institute of General Pathology, Copenhagen.)

CONTENTS.

PAGE
I. The absorption of CO_2 by blood saturated with air or oxygen 244
II. The influence of oxygen and CO on the absorption of CO_2 by blood 252
III. Physiological significance of the results obtained . . . 258
IV. The CO_2 pressure of the mixed venous blood; and the circulation rate 263

I. THE ABSORPTION OF CO_2 BY BLOOD SATURATED WITH AIR.

RECENT investigations have thrown much light on the conditions under which oxygen is absorbed and dissociated by blood; but comparatively little attention has been given of late to the absorption and dissociation of carbon dioxide, although it is now quite evident that this subject is of very great physiological importance.

The existing knowledge was summarised by Bohr in *Nagel's Handbuch der Physiologie*, vol. I., and later by Loewy in *Oppenheimer's Handbuch der Biochemie.* The older account given by Zuntz in *Hermann's Handbuch* is still, however, of great value. Bohr gives at p. 106 a curve, constructed from data obtained by Jacquet and himself from ox-blood and dog's blood, showing the relation between partial pressure of CO_2 and the volume of gas absorbed by the blood (see Fig. 1, below). If we assume this curve to apply approximately to human blood within the living body we are at once confronted by doubt. Considering how exactly the partial pressure of CO_2 is regulated in the alveolar air, and that this pressure is, at least under ordinary resting conditions, that of the arterial blood, as shown definitely by A. and M.

Krogh[1], it does not seem very probable that the resting venous CO_2-pressure differs widely from the arterial: otherwise the exact regulation of the arterial CO_2-pressure would be superfluous, since the pressure of CO_2 in the tissues would vary widely from every slight variation in local circulatory or functional conditions.

On examining Bohr's curve it will be seen that for every five volumes of CO_2 taken up by arterial blood saturated at 40 mm. pressure of CO_2 there is an average rise of about 16·5 mm. of CO_2-pressure. If we make the ordinary assumption that mixed venous blood contains, during rest, about a third less oxygen than arterial blood, it will then have lost about six volumes of oxygen per 100 c.c. of blood, and gained about five volumes of CO_2. The pressure of CO_2 in venous blood would thus be, to judge from Bohr's curve, about 16·5 mm. higher than in arterial blood. This seems rather unlikely in view of the fact that the arterial CO_2-pressure is regulated to considerably less than 1 mm., and 1 mm. of variation will (unless the corresponding variation in $C_{H^\cdot}$ is otherwise compensated) increase or diminish the breathing by about 60 p.c.[2]

Another fact tells much more directly against the assumption that the resting venous CO_2 pressure is as much as 16·5 mm. above the arterial CO_2 pressure. If during rest the breath is held for 20 seconds after a normal expiration the alveolar CO_2-pressure only rises, as a rule, about 6 mm., whereas the calculated rise would be much greater on the assumption that the venous CO_2 pressure is about 16·5 mm. above the normal alveolar CO_2 pressure. To this calculation we shall return later.

We have made a large number of experiments with the object of determining the curve expressing the volumes of CO_2 absorbed at 37° C. by defibrinated human blood at varying pressures of CO_2. The series in which the blood was saturated with mixtures of CO_2 and air will first be described.

For saturating the blood a cylindrical saturating flask of 400 c.c. capacity was used, as in previous experiments of a similar character in the Oxford Laboratory. About 3 or 4 c.c. of blood drawn from a finger, and defibrinated, were poured into the flask, the rubber stopper inserted, and the required volume of CO_2 driven in from a pipette of known capacity. The saturator was then placed in a water-bath at 37·0°, and rotated for about five minutes by means of a motor. The excess of pressure due to the rise of temperature was then released, with the saturator in the bath; and the rotation continued for ten minutes more, though in earlier experiments, referred to below, the period of rotation was longer. The rotation was then stopped and the blood allowed to collect at the lower side of the flask. The flask was then taken out with the neck held downwards so that the blood was collected there, and was wrapped in a warm cloth. A sample of the air was then at once taken into the 10 c.c. burette of a Haldane gas-analysis apparatus through the longer of two narrow bored tubes passing through the rubber stopper; and immediately afterwards about 1 c.c. of blood was sucked out into a 1 c.c. graduated pipette through the shorter tube passing through the stopper. From the graduated pipette exactly 1 c.c. or sometimes 0·7 or 0·5 c.c. was delivered in the ordinary way into the receiving bottle of Brodie's form of the blood gas analysis apparatus described by **Barcroft** and **Haldane**, and the volume of CO_2 per 100 c.c. of blood determined with all the precautions described by **Barcroft** and **Haldane**[1].

---

[1] A. and M. Krogh. *Skand. Arch. f. Physiol.* XXIII. p. 179. 1910.

[2] Campbell, Douglas, Haldane and Hobson. This *Journal*, XLVI. p. 301. 1913.

In estimating from the reading of the gauge the volume of CO_2 given off by the blood it is necessary to allow for the difference in reading produced by a blank experiment. The latter difference is partly due to CO_2 (or some other gas) given off by the ammonia and ferricyanide solutions used for getting rid of the oxygen, and partly to differences in vapour tension caused by the addition of the tartaric acid. We found that if the ammonia solution has been exposed to air, or the ferricyanide is not freshly made and pure, they may give off an appreciable volume of gas, so we always used freshly made solution, and also took care that the ammonia and distilled water used for making the dilute ammonia solution were free from CO_2, and were prevented from absorbing it. The analyses were also made at a place where the air was quite pure. In making the blank experiments ·75 p.c. salt solution (free of CO_2) was placed in the apparatus in order to represent the blood, and saponin was also added, just as in an ordinary determination. With these precautions the correction furnished by the blank experiment was extremely small, and might be either negative or positive. The readings of the gauge were usually about 120 to 150 mm., and the correction seldom more than 1 or 2 mm.

In our first experiments several determinations were made successively with the same sample of blood at varying pressures of CO_2. The shape of the curve obtained on plotting the results aroused our suspicions at once. At first we thought that there were probably inaccuracies in the method of blood-gas analysis; but further investigation showed that the blood in the saturator was undergoing progressive alteration. Thus the first determination of a series with the same sample of blood was made at 40·4 mm. CO_2-pressure, and gave 51·7 vols. of CO_2 per 100 c.c. of blood. After five hours, during which a series of determinations had been made at other pressures, a second determination was made at about 40 mm., and gave only 41·2 vols.

In order to gain some more definite idea of the rate at which this change occurs in defibrinated blood kept at the body temperature we made several successive determinations on the same blood at about the same pressure of CO_2. The results were as follows:

		Vol. of CO_2 per 100 c.c. of blood	Pressure of CO_2 in mm. of Hg.
No. 1.	Blood in saturator at 37° for 19 minutes	50·7	38·7
No. 2.	,, ,, ,, 54 ,,	49·2	41·0
No. 3.	,, ,, ,, 137 ,,	46·0	40·8

---

[1] This *Journal*, XXVIII. p. 232. 1902.

By reference to the curve (Fig. 1) the results can be corrected for the slight differences in CO_2-pressure in the different determinations. It then appears that between the first and second determinations (reckoning both as at 41 mm.) there was a drop of 2·6 vols. in the CO_2 absorbed by the blood after 35 mins. exposure at 37°: while between the second and third, during 83 mins. exposure, there was only a drop of 3·1 vols. This indicated that the change is probably not due to bacterial action; but we have not followed the matter further.

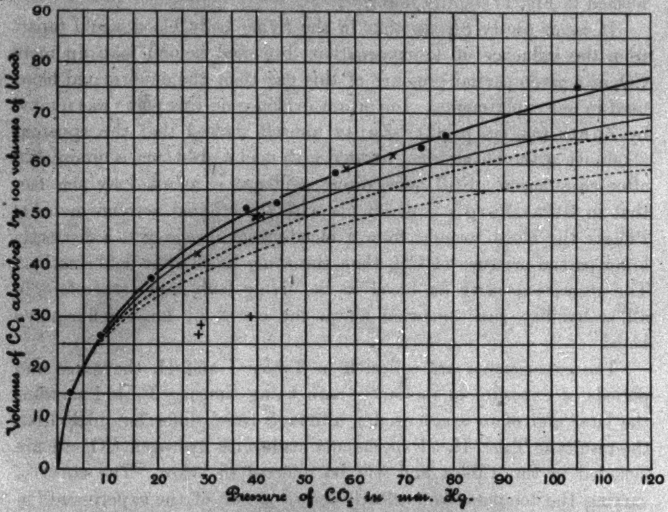


Fig. 1. Upper continuous line—absorption of CO_2 by blood of J. S. H. in presence of air and CO_2.

Lower continuous line—ditto after deducting the CO_2 in solution.

Upper dotted line—absorption of CO_2 by blood of ox and dog (Bohr's data) in presence of air and CO_2.

Lower dotted line—ditto after deducting CO_2 in solution.

× Absorption of CO_2 in blood of C. G. D. in presence of air and CO_2 during rest.

+ Absorption of CO_2 in blood of C. G. D. in presence of air and CO_2 just after violent exercise.

It was clear that in order to get concordant results a fresh sample of blood must be taken for each determination, and kept at 37° for as short a time as possible, and for about equal periods in different determinations. As soon as this precaution was taken we obtained results which, on plotting, gave a definite and concordant curve (see Fig. 1). In the earlier determinations we had only one tube passing through the stopper of the saturator. For this reason we took out the air sample first, before the pressure due to the warming of the saturator had been released; and the saturator was then returned to the bath, the pressure released, and the rotation continued for at least ten minutes more. The total time in the bath was therefore longer than in the later experiments, and this made the results (which are not plotted in Fig. 1) slightly lower.

It seems pretty certain that in the living body blood would (apart from the influence of de-oxygenation, discussed below) take up more CO_2 at a given partial pressure of this gas than the defibrinated blood used in our experiments. Important evidence on this point was quoted by Zuntz (*op. cit.* p. 82). Zuntz himself showed that the apparent alkalinity of the blood, when titrated with acid and litmus, is diminished after coagulation. Still more direct evidence is afforded by the fact that in Strassburg's experiments with the original aerotonometer of Pflüger the blood straight from a blood-vessel always gave a distinctly lower partial pressure of CO_2 than the same blood after defibrination[1]. The absorption curve for blood in the living body would therefore, in all probability, run somewhat above the curve for fresh defibrinated blood.

The experiments are collected in Tables I and II, the dates and periods of exposure in the water-bath being shown. Table I contains the later and more exact results, although these differ but little from the results in Table II. Experiments made with hydrogen, CO, etc. are included in the Tables, and will be referred to below. The effect of varying the temperature is clearly shown in one of the experiments in Table II.

The later and more exact data for the blood of J. S. H and C. G. D. are plotted in Fig. 1, from which it will be seen that the results for the same individual from day to day lie very close to the curve shown in the figure. This indicates that under ordinary conditions the blood of an individual is very constant as regards its capacity for absorbing CO_2. The slight variations from the curve might well be due to small experimental errors, although it seems probable *a priori* that real variations of appreciable amount occur from hour to hour, or from day to day.

The results for the blood of C. G. D. are slightly different from those for J. S. H. On the other hand those for J. C. are practically the same as those for J. S. H., as shown in Table II. Several isolated observations on blood from other persons are also given in Table I. On comparing these results with the curve it will be seen that at about 40 mm. CO_2 pressure the blood of some persons gave slightly less, and of others

---

[1] Strassburg. *Pflüger's Archiv*, VI. p. 65.

TABLE I. *Later Results.*

Date	Subject	Time at 37° before analysis, in minutes	CO_2-pressure in saturator, mm. of Hg.	Volumes of CO_2 per 100 volumes of blood	Percentage of O_2 in saturator	Remarks
20/1/14	J. S. H.	20	18·9	37·1	—	Air + CO_2 in saturator.
,,	,,	21	17·9	41·6	0·26	H_2 ,, ,,
,,	,,	20	78·5	65·7	—	Air ,, ,,
,,	,,	19	78·7	75·0	0·26	H_2 ,, ,,
21/1/14	,,	20	37·7	51·2	—	Air ,, ,,
,,	,,	21	37·9	56·1	0·21	H_2 ,, ,,
,,	,,	20	8·7	26·3	—	Air ,, ,,
,,	,,	21	7·75	30·8	0·28	H_2 ,, ,,
,,	,,	20	8·1	30·6	0·47	H_2 ,, ,,
26/1/14	,,	18½	56·1	58·3	—	Air ,, ,,
,,	,,	20	58·2	64·5	0·27	H_2 ,, ,,
,,	,,	18½	2·15	15·4	0·74	H_2 ,, ,,
,,	,,	20	2·6	15·1	—	Air ,, ,,
27/1/14	,,	23	105·2	75·3	—	Air ,, ,,
,,	,,	19	111·1	82·0	0·36	H_2 ,, ,,
28/1/14	,,	21	73·3	63·1	—	Air ,, ,,
,,	,,	21	74·2	70·4	0·32	H_2 ,, ,,
,,	,,	19	41·7	51·1	91·2	O_2 ,, ,,
,,	,,	19	44·1	52·4	—	Air ,, ,,
29/1/14	,,	22	37·7	48·9	0·30	$H_2 + CO_2 + 2·3\,\%$ of CO.
2/2/14	C. G. D.	20	58·2	59·2	—	Air + CO_2 ,, ,,
,,	,,	20	57·5	63·4	0·31	H_2 ,, ,, ,,
,,	,,	17	40·9	49·8	—	Air ,, ,, ,,
,,	,,	19	67·6	61·8	—	Air ,, ,, ,,
4/2/14	,,	20	39·6	49·4	—	Air ,, ,, ,,
,,	,,	21	38·8	30·1	—	Air + CO_2 5½′ after violent exertion.
7/2/14	,,	22	28·0	42·5	—	Air + CO_2.
,,	,,	18½	28·8	28·7	—	Air + CO_2 7′ after violent exertion.
9/2/14	,,	20	39·4	49·5	—	Air + CO_2.
,,	,,	20	28·2	26·9	—	Air + CO_2 6½′ after violent exertion.
,,	,,	18	35·3	47·0	—	Air + CO_2 77′ after violent exertion.
4/2/14	J. G. P.	21	39·7	51·0	—	Air + CO_2.
,,	J. M. C.	22	38·2	45·7	—	Air ,,
9/2/14	S.	22	38·3	47·4	—	Air ,,
,,	C. W. A.	20	40·3	48·5	—	Air ,,

TABLE II. *Earlier Results.*

Date	Subject	Time at 37° before analysis, in minutes	CO_2-pressure in saturator, mm. of Hg.	Volumes of CO_2 per 100 volumes of blood	Percentage of O_2 in saturator	Remarks
6/8/13	J. S. H.	55	3·1	13·9	—	Air + CO_2 in saturator.
7/8/13	,,	50	19·2	36·7	—	Air ,, ,,
10/8/13	,,	47	40·4	51·7	—	Air ,, ,,
11/8/13	,,	43	74·1	64·1	—	Air ,, ,,
,,	,,	38	117·1	79·2	—	Air ,, ,,
,,	,,	45	30·8	45·6	—	Air ,, ,,
,,	,,	45	208·3	94·5	—	Air ,, ,,
12/8/13	,,	—	21·1	60·8	—	Air + CO_2. Temp. 17·9°. Saturated 30′.
,,	,,	35	11·6	30·3	—	Air + CO_2 in saturator.
23/9/13	,,	37	33·7	47·8	—	Air ,, ,,
,,	,,	37	34·5	51·4	0·43	H_2 ,, ,,
,,	,,	40	59·8	57·9	—	Air ,, ,,
,,	,,	23	60·9	63·2	0·42	H_2 ,, ,,
24/9/13	,,	35	60·6	64·2	0·26	H_2 ,, ,,
26/9/13	,,	35	51·0	53·2	—	Air ,, ,,
,,	,,	35	53·0	54·5	—	Air ,, ,,
,,	,,	35	52·3	60·9	0·48	H_2 ,, ,,
27/9/13	,,	35	52·5	56·5	—	Air ,, ,,
,,	,,	35	51·4	55·2	90·0	Oxygen ,, ,,
,,	,,	40	52·1	58·8	3·61	$H_2 + O_2 + CO_2$,, Hb 40 % saturated with O_2.
13/1/14	,,	35	41·5	49·7	—	Air + CO_2 in saturator.
,,	,,	35	42·4	55·9	0·25	H_2 ,, ,,
8/8/13	C. G. D.	55	3·9	16·4	—	Air ,, ,,
11/8/13	,,	43	41·0	47·6	—	Air ,, ,,
12/8/13	,,	55	79·0	63·3	—	Air ,, ,,
,,	,,	46	18·1	34·5	—	Air ,, ,,
,,	J. C.	43	42·1	50·1	—	Air ,, ,,
,,	,,	33	82·3	66·7	—	Air ,, ,,

slightly more than 50 volumes of CO_2; but the general agreement is very striking.

Our curve differs from that given by Bohr from his and Jaquet's observations on ox and dog blood. It will be seen that whereas the blood of J. S. H. contains 51·5 vols. of CO_2 at 40 mm. CO_2-pressure, and takes up 15 vols. in rising to 80 mm., ox and dog blood seem only to contain 46 vols. at 40 mm. CO_2-pressure, and to take up 15 vols. in rising from 40 to 89 mm. CO_2-pressure. The data on which Bohr's curve was constructed are, however, rather scanty, considering that the samples were from different animals.

We have plotted, not only the absorption curves as actually determined, but also the dissociation curves calculated on Bohr's data for the co-efficient of solubility of CO_2 in blood at body temperature. The conditions determining chemical combination of CO_2 in the blood, are, however, so complex that we shall not attempt to give a chemical interpretation of the dissociation curve.

It might be suspected that a relation would exist between the absorption curve for CO_2 in blood and the normal resting alveolar CO_2-pressure of the same individual. Our data show, however, that there is no such relation. Thus the alveolar CO_2-pressure of J. C. is about 5 to 6 mm. lower than that of J. S. H., but the absorption curves are hardly distinguishable. In J. M. C., whose blood indicated the lowest absorption curve, the alveolar CO_2 was nearly the same as in J. S. H. Hence a low normal alveolar CO_2-pressure is not the compensating counterpart of a low absorption curve. The subject will be further discussed below in connection with the venous CO_2-pressure.

For the same individual under different physiological conditions we have obtained very striking evidence of the relation between resting alveolar CO_2-pressure and the absorption curve. It was found by **Douglas** and **Haldane**[1] that after a severe muscular exertion, such as running quickly up-stairs, the resting alveolar CO_2-pressure is lowered markedly, and takes about an hour to return to normal. This was attributed to the presence in the blood of lactic acid produced during the severe exertion, owing to the blood-supply being insufficient to supply the muscles with the oxygen required. The actual presence of lactic acid in the blood during the period of lowered alveolar CO_2-pressure was shortly afterwards demonstrated by **Ryffel**[2]. We have made the following experiments with C. G. D. as subject on the effect of severe exertion on the absorption curve and alveolar CO_2-pressure:

Exp. I. Before exertion alveolar CO_2-pressure = 38·2 mm. Blood in presence of air and 39·6 mm. of CO_2-pressure absorbed 49·4 vols. of CO_2.

The exertion consisted of running thrice as quickly as possible up 40 feet of stairs, and down again. After a rest of five minutes this was repeated; and after another rest of five minutes the stairs were again ascended twice. The exertion caused much panting. A sample of blood was taken 5 to 8 mins., and the alveolar samples 10 and 14 mins. after the last ascent.

After exertion alveolar CO_2-pressure = 29·4 mm. Blood in presence of air and 38·5 mm. of CO_2-pressure absorbed 30·1 vols.

Alveolar CO_2-pressure 1½ hours after the exertion = 38·5 mm.

Exp. II. Before exertion alveolar CO_2 = 39·3 mm. Blood in presence of air and 28·0 mm. of CO_2-pressure absorbed 42·5 vols. of CO_2.

---

[1] This *Journal*, XXXVIII. p. 431. 1909.

[2] *Ibid.* XXXIX. *Proc. Physiol. Soc.* p. xxix. 1901.

The exertion consisted of running twice, as quickly as possible, up and down the same stairs. This was repeated five times within 15 mins., including a rest of about 3 mins. after each exertion. Blood sample about 7 mins., and alveolar samples 10 and 15 mins. after last exertion.

After exertion alveolar CO_2=31·8 mm.: blood in presence of air and 28·8 mm. of CO_2-pressure absorbed 28·7 vols. of CO_2.

EXP. III. Before exertion alveolar CO_2=38·1 mm. Blood in presence of air and 39·4 mm. of CO_2-pressure absorbed 49·5 vols. of CO_2. Hb of sample=101 °/₀.

The exertion consisted in running thrice, as quickly as possible, up and down the same stairs. This was repeated four times within 14 mins., including rests. Blood sample about 6 mins., and alveolar samples 10 and 14 mins. after the exertion.

After exertion alveolar CO_2=31·3 mm.: blood in presence of air and 28·2 mm. of CO_2-pressure absorbed 26·9 vols. of CO_2.

Further alveolar samples were taken 67 and 71 mins., and a blood-sample 78 mins. after the exertion. Alveolar CO_2=35·5 mm.: blood in presence of air and 35·3 mm. of CO_2-pressure absorbed 47·0 vols. of CO_2.

The results of the blood analyses in these experiments are indicated by crosses in Fig. 1. It will be seen that the presence of the lactic acid in the blood has led to an enormous temporary alteration in the absorption curve. Corresponding with this there is a reduction of about a fifth in the alveolar CO_2-pressure. Within little over an hour, however, the absorption curve had returned to about normal, corresponding with the return of the resting alveolar CO_2-pressure to nearly normal. Corresponding differences in the normal absorption curve will probably be discovered under various pathological or compensatory conditions of "acidosis."

The existence of a relatively constant absorption or dissociation curve of CO_2 for the blood of an individual, or a species, furnishes fresh evidence pointing to the exactness of the physiological regulation of the composition of the blood. The disturbing conditions under which blood-samples are usually obtained from animals have hitherto prevented the recognition of this fine regulation.

II. THE INFLUENCE OF OXYGEN AND CO ON ABSORPTION OF CO_2 BY BLOOD.

It appears to have first been suggested by Ludwig that oxygen might have some influence in driving out CO_2 from its combinations in the blood. Experiments under Ludwig's direction by Holmgren seemed to confirm this suggestion; but Ludwig himself concluded, later, that any such influence is, if it exists, very small[1]. The lung catheter and aerotonometer experiments made by Wolffberg and

---

[1] *Wiener Med. Jahrb.* p. 169. 1865.

Strassburg in Pflüger's laboratory indicated that the tension of CO_2 in venous blood is about the same as in the blocked alveoli; and by those, at any rate, who accept the theory that there is no active excretion of CO_2 by the lung epithelium, these experiments have been accepted as evidence that the venous CO_2 tension is never lower than that of the alveolar air. In lung-catheter experiments, however, the oxygenation of the venous blood is completely arrested in the blocked alveoli, so that the experiments prove nothing as regards the point in question.

The matter is discussed by **Bohr** (*op. cit.* pp. 106–7) and he quotes experiments which bear directly on the subject. These experiments show no distinct influence of oxygen on the absorption of CO_2; but all the data are for very low pressures of CO_2—under 9 mm. The discovery made by **Bohr** and his pupils that CO_2 helps to expel oxygen from its combination with hæmoglobin would naturally point to the probability of oxygen having a corresponding influence in turning out CO_2; but in consequence of the evidence brought forward by **Bohr** himself to the contrary, the view that oxygen is without influence in this direction has been generally accepted. **Loewy** however, in view of the significant results of his experiments on the influence of oxygen on the osmotic pressure of blood[1], maintains that oxygen must have some influence.

The data quoted by **Bohr** were obtained in the course of the well-known investigations by **Hasselbalch**, **Krogh** and himself on the influence of CO_2 on the dissociation of oxygen from blood[2]. In this paper the authors remark that "no certain action of O_2-pressure on the simultaneous absorption of CO_2 was observed. If any such action exists it is at any rate small and can have no special significance for the conditions in the living body." In a footnote to a recent paper by **Lundsgaard** and himself on the reaction of the blood[3], **Hasselbalch** remarks, however, that reconsideration of the notes of these experiments has convinced him that with the same CO_2-pressure blood takes up a little more CO_2 with a low than with a high O_2-pressure; and he quotes figures showing the differences, as calculated by interpolation. The figures quoted by **Bohr** permitted of direct comparisons without interpolation; but it seems certain from our own results that errors had crept in, owing, perhaps, to too long a time having elapsed between the determinations.

---

[1] **Loewy.** *Berl. klin. Wchnsch.* No. 2, 1903; also p. 58 of his section of *Oppenheimer's Hdb. d. Biochemie*, 1908.

[2] *Skand. Arch. f. Physiol.* XVI. p. 411. 1904.

[3] *Biochem. Zeitsch.* XXXVIII. p. 88. 1912.

In order to gain information bearing on venous CO_2 tension we had made a number of experiments on the rise of CO_2-pressure in the alveolar or expired air when the breath is held, or a very small volume of air is continuously re-breathed. In some of these experiments oxygen was used, as **Hill** and **Flack** have shown that when oxygen is breathed the breath can be held, and re-breathing can be continued, for much longer periods than with air[1]. It was observed that not only is this the case, but that during a given period the CO_2 percentage in the alveolar or re-breathed air goes higher when any marked fall in the normal alveolar oxygen percentage is prevented.

The following series will illustrate this point.

Subject J. C. Before each experiment a Krogh spirometer[2] was filled with exactly 2·5 litres of either air or oxygen. At the end of an ordinary resting expiration the subject started breathing from and into the spirometer. At the end of 2 to 3½ mins. a sample of alveolar air was taken and analysed. The experiments were made in pairs, one after the other, the experiment with oxygen being sometimes made first, and sometimes second. The results are shown in Table III.

TABLE III. *Composition of alveolar air after re-breathing 2·5 litres of air or oxygen. Subject J. C.*

Date	Duration of re-breathing, in minutes	Air re-breathed		O_2 re-breathed CO_2 per cent.
		O_2 per cent.	CO_2 per cent.	
1/11	2	—	7·15	7·48
	2½	—	7·50	7·70
	2½	—	7·34	7·85
8/1	3	6·6	6·90	7·80
	2½	7·9	7·20	7·60
9/1	3½	4·9	7·95	8·16
12/1	3	6·1	7·20	8·01
	3	5·2	7·35	7·91
Mean	...	...	7·32	7·81

It will be seen that in every case without exception the percentage of CO_2 was higher when oxygen was re-breathed, the average difference being about 0·5 p.c. In many other less exact experiments a similar result had been obtained.

---

[1] This *Journal*, XXXVII. p. 77. 1908: see also **Douglas** and **Haldane**, *Ibid.* XXXVIII. p. 424.

[2] **Krogh** and **Lindhard**. *Skand. Arch. f. Physiol.* XXVIII. p. 23. 1912.

In another series with J. S. H. as subject, the alveolar air was analysed after holding the breath for 10 secs. from expiration, (*a*) after ordinary air had been breathed, and (*b*) after six normal breaths had been taken from a bag of hydrogen. No hyperpnœa was produced by the hydrogen before the six breaths had been taken. The results are shown in Table IV.

TABLE IV. *Composition of alveolar air after holding the breath for 10 seconds.*

After normal breathing of air		After six normal breaths of H_2	
O_2 p.c.	CO_2 p.c.	O_2 p.c.	CO_2 p.c.
12·25	6·04	—	—
—	6·26	6·10	5·95
—	6·22	6·42	6·19
—	6·04	6·27	5·80
—	6·14	6·82	5·94
—	6·24	6·21	6·01
Mean	6·18	—	5·98

In this series it is also quite clear that marked deficiency in the alveolar oxygen percentage is associated with a diminished rise in the alveolar CO_2 percentage.

The fact that the oxygen percentage of the alveolar air has such a distinct influence on the amount of CO_2 given off by the lungs led us to suspect that some experimental fallacy may have been present in Bohr's experiments. We accordingly made a series of experiments to ascertain whether oxygenation of the hæmoglobin has not, after all, a considerable influence on the capacity of the blood for absorbing CO_2.

The experiments were made at various pressures of CO_2, and in precisely the same manner as the experiments embodied in Fig. 1; the only difference being that the saturator was filled with hydrogen in place of air, so that the hæmoglobin was practically speaking completely reduced. In each experiment the oxygen, as well as the CO_2, in the gas used was determined. Usually only about 0·3 p.c. of oxygen was present; and reference to the dissociation curve of oxy-hæmoglobin in human blood shows that this small proportion of oxygen would be insufficient to saturate the hæmoglobin to the extent of more than about 2 p.c. The results are shown in Tables I and II, and graphically in Fig. 2.

It will be seen that in every case the volume of CO_2 absorbed by blood at a given partial pressure of this gas is considerably greater when the blood is reduced than when the hæmoglobin is saturated with oxygen. At the physiologically important part of the curve, between 35 and 60 mm. pressure of CO_2, the extra amount of CO_2 taken up is about 5 to 6 vols. per 100 vols. of blood.

To ascertain whether the influence of oxygen in diminishing the CO_2-carrying capacity of the blood coincides with the degree of saturation of the hæmoglobin, we have made several further experiments. In the first place we have compared, in samples from the same subject and within an hour of one another, the CO_2-carrying power of the blood when pure oxygen and when ordinary air were used in filling the saturator before the CO_2 was added. The results were that with air and 41·7 mm. of CO_2-pressure the blood took up 51·1 vols. of CO_2, while with oxygen and 44·1 mm. of CO_2-pressure it took up 52·4 vols. If the latter result is corrected (by means of the curve in Fig. 1) to correspond with the CO_2-pressure in the first experiment, the result is that with 41·7 mm. of CO_2-pressure the blood took 51·1 vols., and with oxygen 51·3 vols.

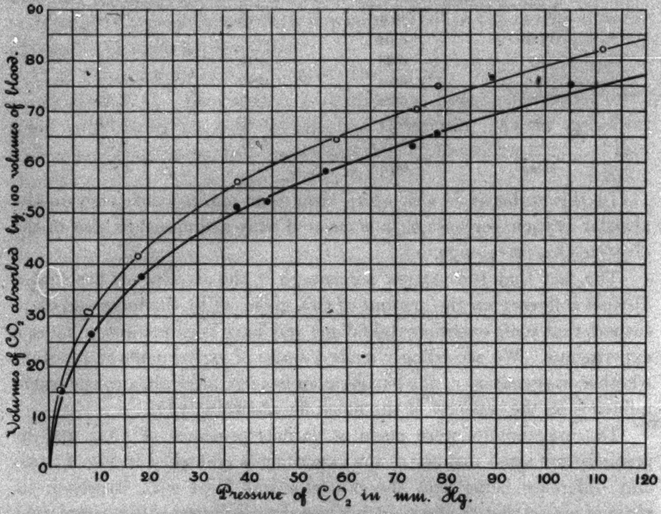


Fig. 2. Lower curve—absorption of CO_2 by blood of J. S. H. in presence of air and CO_2. Upper curve—absorption of CO_2 by blood of J. S. H. in presence of hydrogen and CO_2.

With the air mixture the oxygen-pressure was 141 mm., and with the nearly pure oxygen about 660 mm. With air the saturation of the hæmoglobin would be 98 p.c., so that the oxygen would practically speaking have no further effect in saturating the hæmoglobin. The fact that the oxygen had no effect in diminishing the CO_2 absorbed by the blood points to connection between the diminished CO_2-carrying capacity and saturation of the hæmoglobin with oxygen. An earlier, but less exact experiment of the same kind at 52 mm. of CO_2-pressure, gave a similar result.

In a further experiment the saturator contained 3·61 p.c. of oxygen (25·8 mm. oxygen-pressure), and the hæmoglobin was found to be 40 p.c. saturated. The blood absorbed 58·8 vols. of CO_2, while the mean of two previous experiments on the same day with air and oxygen in the saturator gave 55·9 vols. for the same CO_2-pressure. The extra CO_2 taken up in the second sample was 2·9 vols., as compared with 5·5 vols. when the blood is completely reduced. Hence the increase of CO_2-carrying capacity runs parallel, not with the partial pressure of oxygen, but with the reduction of the hæmoglobin. The hæmoglobin was 60 p.c. reduced, and the increased CO_2 capacity was 53 p.c. of the full increase. The correspondence is as close as could be expected, considering the experimental errors.

Further evidence of the connection between the saturation of the hæmoglobin and the effect on the CO_2-carrying capacity was furnished by an experiment in which the blood was saturated with CO in the practical absence of oxygen. The saturator was first filled with hydrogen, and 2·3 p.c. of CO added, besides CO_2. The oxygen percentage was 0·30, and the CO_2-pressure 37·7 mm. The blood (of J. S. H.) gave 48·9 vols. p.c. of CO_2. This is slightly below the average amount for this pressure of CO_2 with the blood of J. S. H.; hence in spite of the absence of oxygen there was no increase in the volume of CO_2 absorbed. The blood was of course completely saturated with CO, as far more CO was present than was needed for saturation.

It is well known that hæmoglobin, in common with other proteins, plays the part of a weak acid in driving out CO_2 from its combinations with alkali in the blood. Pflüger[1] showed that not only can all the combined CO_2 in whole blood be expelled easily by pumping, but that the CO_2 of added sodium carbonate can also be expelled; whereas the CO_2 can only be partly pumped out of a pure sodium bi-carbonate solution, or out of serum, by an ordinary blood-pump. It appears from our experiments that oxy-hæmoglobin or CO-hæmoglobin has a greater influence than an equal proportion of reduced hæmoglobin in expelling CO_2. It may be that the oxy-hæmoglobin molecule is more acid. Another possible explanation is that when oxy-hæmoglobin in blood is reduced the molecules aggregate to a greater extent, in accordance with the hypothesis put forward by Douglas, J. S. Haldane and J. B. S. Haldane[1]. The aggregated molecules would naturally have less influence in expelling CO_2. The slow change which, as described above, occurs in blood when it is kept at 37° may also be due to a gradual autolysis with breaking down of the complex molecules or molecular aggregates. It is evident, however, that further experiments are needed for the elucidation of the many questions connected with the manner in which CO_2 is combined in blood.

---

[1] Pflüger. *Ueber die Kohlensäure des Blutes.* Bonn, 1864.

III. PHYSIOLOGICAL SIGNIFICANCE OF THE RESULTS OBTAINED.

As the average oxygen capacity of the hæmoglobin in human blood is 18·5 c.c. per 100 c.c. of blood, and the hæmoglobin in arterial blood is 96 p.c. saturated, average arterial blood contains 17·76 c.c. of oxygen in combination. Adding to this ·33 c.c., which will be in simple solution at the oxygen pressure of arterial blood, the total is 18·1 c.c. If the whole of this oxygen were exactly used up in the tissues, about 15 c.c. of CO_2 would be formed, and the venous blood would therefore contain an excess of 15 c.c. of CO_2. According to the curve shown in Fig. 1 the pressure of CO_2 would, with an excess of CO_2 of 15 vols., rise from 40 to 80 mm. This, it may be remarked, is less than would appear to be the case from Bohr's curve, which would show a rise of pressure from 40 to 87 mm.

In the passage of the blood through the tissues the oxy-hæmoglobin would, however, be reduced, and the CO_2-pressure would therefore, as a matter of fact, rise along the thick line joining the two curves shown in Fig. 3. The pressure of CO_2 would therefore only rise 22 mm. So far as is known the oxygen in venous blood is never completely used up during life; and it is usually inferred from the available data that the blood does not on an average lose more than about a third of its oxygen in passing round the circulation during rest. In this case the rise of CO_2-pressure would be reduced to 7 mm., which would render much more intelligible the exactness with which the alveolar CO_2-pressure is regulated. The actual average rise is only about 5 mm., as will be shown below.

---

[1] This *Journal*, XLIV. p. 296. 1912.

As was recently pointed out by **Campbell, Douglas, Haldane** and **Hobson**[1], the available evidence, and particularly the experiments of **Hasselbalch**[2], point to a very exact regulation of the hydrogen ion concentration ($C_{H^{\cdot}}$) of the arterial blood. The reduction of hæmoglobin, in increasing the CO_2-carrying power of the blood, must also reduce the difference in $C_{H^{\cdot}}$ between arterial and venous blood. With the existing methods it seems scarcely possible to measure the difference in $C_{H^{\cdot}}$ between oxygenated and reduced blood; but we may provisionally assume that with equal differences of CO_2-pressure oxygenated and reduced blood give about equal differences in $C_{H^{\cdot}}$. It also seems

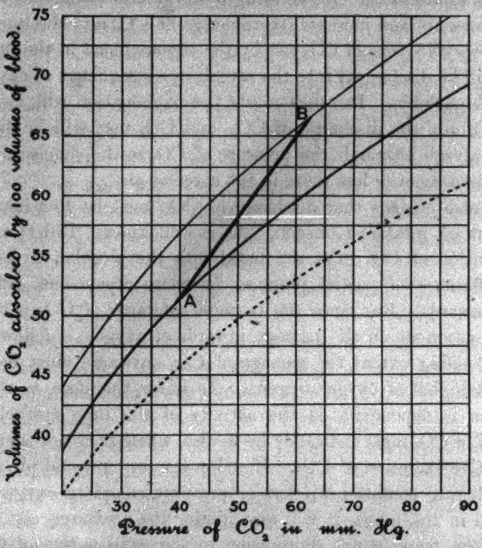


Fig. 3. Upper curve—absorption of CO_2 by blood of J. S. H. in presence of hydrogen and CO_2.

Middle curve—absorption of CO_2 by blood of J. S. H. in presence of hydrogen and CO_2.

Lower curve—absorption of CO_2 in blood of ox and dog in presence of air and CO_2 (**Bohr**'s data).

The thick line *A—B* represents the absorption of CO_2 by the blood of J. S. H. within the body.

[1] This *Journal*, XLVI. p. 301. 1913.

[2] *Biochem. Ztschr.* XLVI. p. 428. 1912.

probable that the $C_{H\cdot}$ of reduced blood is somewhat less, at a given pressure of CO_2, than that of oxygenated blood: in which case the difference in $C_{H\cdot}$ between arterial and venous blood will be somewhat less than what corresponds to 5 mm. of CO_2-pressure for either oxygenated or reduced blood. It is doubtless the $C_{H\cdot}$ of the venous blood leaving the respiratory centre and kidneys that is of immediate importance in the general regulation of the $C_{H\cdot}$ of the blood as a whole; and it is of interest to know that the $C_{H\cdot}$ of venous blood must be so near to that of arterial blood.

On the return of the blood to the lungs the oxygenation of the blood will diminish the CO_2-carrying power of the blood, and thus raise its CO_2-pressure and increase its discharge of CO_2 into the lung alveoli. Out of every 15 vols. of CO_2 lost by the venous blood in the lungs, 5 to 6 vols. will be discharged into the alveoli in consequence of oxygenation of the hæmoglobin. In other words the oxygenation will increase by at least 50 p.c. the discharge of CO_2, and CO_2 may still be discharged into the alveoli although the pressure of CO_2 in the venous blood is no greater than, or even less than, in the alveolar air.

It is also evident that discharge of CO_2 must be to a large extent dependent on intake of oxygen. As is well known, Bohr[1] calculated that diffusion of CO_2 from the blood into the alveolar air is, with a given difference of partial pressure, far more rapid than diffusion of oxygen inwards. But since the passage outwards of CO_2 is to a large extent consequent on the passage inwards of oxygen, it follows that to a corresponding extent the passage of CO_2 outwards must be slightly slower than that of oxygen inwards. In so far, therefore, as absorption of oxygen is dependent on the activity of the lung epithelium the giving off of CO_2 must indirectly be so also, whether or not there is ever direct active excretion of CO_2. If, moreover, any unusual physiological or pathological condition hinders or prevents complete oxygenation of the blood in the lungs, it will also hinder the discharge of CO_2; and, other things being equal, diminution in the oxygenation of the blood will always be accompanied by increase of its CO_2-percentage, unless this increase is compensated by increase in the breathing.

On examining the literature bearing on the influence of oxygen on the absorption of CO_2 by blood either inside or outside the body we have discovered a number of observations which seem to have now found their definite explanation. There seems to be little doubt that Holmgren's results (referred to above) were really due to the action of oxygen in expelling CO_2, and that in Ludwig's later experiments the blood-gas methods were too imperfect to show the effects. There were, however, isolated observations by Preyer[1] and others showing that in presence of oxygen CO_2 is more quickly expelled from blood than in the absence of oxygen. Wolffberg, also, in his second series of lung-catheter experiments, found that oxygen makes a difference[2]; and the more recent observations of Loewy[3], showing that the osmotic pressure in blood saturated with CO_2 is more quickly lowered by a stream of oxygen than by hydrogen or nitrogen, point in the same direction. In 1892 Werigo[4] in a very interesting paper returned to the line of experiment initiated in Holmgren's paper, and showed that when one lung was kept distended with oxygen, and the other with hydrogen, the percentage of CO_2 was always higher (by about 2 or 3 p.c. on an average) in the oxygen lung. The period of the distension varied from half a minute to half an hour. From his experiments Werigo concluded that oxygen helps to expel CO_2 from the blood; but he did not verify this on blood outside the body, and Bohr's later experiments with blood outside the body gave apparent negative results, as mentioned above. Bohr himself[5] attributed Werigo's results to oxidation occurring in the blood passing through the oxygen lung, and not in the hydrogen lung. He gave the same explanation of a series of results obtained in his laboratory by Halberstadt (not published elsewhere than in Bohr's article p. 207). Halberstadt found that when a rabbit was made to breathe hydrogen (containing less than 4 p.c. of oxygen) through one lung, and air through the other, the amount of CO_2 given off by the hydrogen lung was about 30 p.c. less. This result also is attributed by Bohr to the absence of oxidation in the lungs, but it is just the result which might be expected in view of the results of our experiments; and recent experiments by Evans and Starling[6], and also by Henriques himself[7], show that the conclusions drawn from Bohr and Henriques' experiments, which seemed to indicate very considerable oxidation in the lungs, were mistaken.

---

[1] Bohr. *Skand. Arch. f. Physiol.* XXII. p. 261. 1909.

The data embodied in the thick line in Fig. 3, when taken in conjunction with existing data on the influence of CO_2 on the dissociation curve of the oxy-hæmoglobin or CO-hæmoglobin in whole blood, make it possible to calculate approximately the influence of CO_2 on the dissociation curve of oxy-hæmoglobin in the living body. If the blood were completely reduced, and a corresponding formation of CO$_2$ occurred, the rise of CO$_2$-pressure would only amount to 22 mm., as shown in Fig. 3, and the actual rise of CO$_2$-pressure in mixed venous blood during rest is only about 5·3 mm., as shown below. From our own data and those of Barcroft and Poulton[1] we have constructed the curves in Fig. 4, showing the dissociation curves of oxy-hæmoglobin in presence of a constant pressure of 40 mm. of CO$_2$ (thick line) and in presence of the increasing CO$_2$-pressure as the blood passes through the tissues (thin line). It will be seen that the influence of CO$_2$ on the dissociation of oxy-hæmoglobin in the living body is but slight. The influence of oxygen on the dissociation of CO$_2$ seems to be of much greater physiological importance than the influence of CO$_2$ on the dissociation of oxygen. In hydræmic conditions, CO poisoning, etc., there must be considerable disturbance of the normal regulative influence of hæmoglobin on the CO$_2$-pressure of the circulating blood, and this factor must enter into the symptom-complex.

---

[1] *Stzngsb. d. Wiener Akad.* XLIX. p. 27.

[2] *Pflüger's Archiv*, VI. p. 28. 1872.

[3] *Berl. klin. Wchnsch.* No. 2. 1903.

[4] *Pflüger's Archiv*, LI. p. 321. 1892.

[5] *Nagel's Hdb.* I. p. 208.

[6] This *Journal*, XLVI. p. 413. 1913.

[7] *Biochem. Ztschr.* LVI. p. 230. 1913.

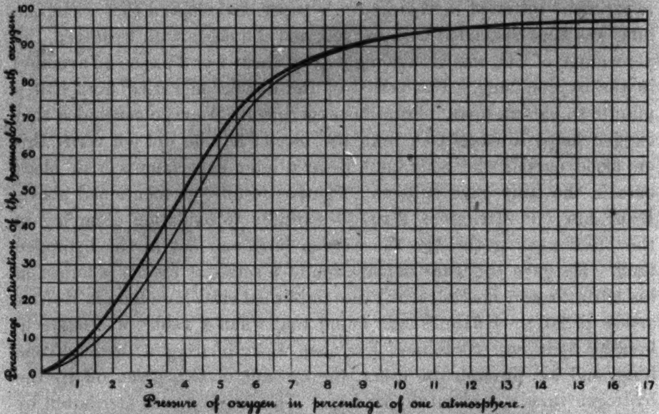


Fig. 4. The thick line shows the dissociation curve of oxy-hæmoglobin in the blood of J. S. H. and C. G. D. in the presence of 40 mm. pressure of CO$_2$. The thin line represents the dissociation curve of oxy-hæmoglobin in the blood of J. S. H. and C. G. D. within the body.

---

[1] This *Journal*, XLVI. *Proc. Physiol. Soc.* p. iv.

IV. THE PRESSURE OF CO_2 IN THE MIXED VENOUS BLOOD; AND THE CIRCULATION RATE.

It is evident that if we can experimentally determine the pressures of CO_2 in the arterial and mixed venous blood we can deduce from the absorption curve of blood for CO_2 the extra amount of CO_2 in the venous blood. From this and the respiratory exchange we can also deduce the volume of blood passing through the lungs. If we also know the amount of oxygen in the arterial blood we can deduce the amount in the venous blood, and, with the help of the dissociation curve of the oxy-hæmoglobin of the blood, the oxygen pressure of the venous blood. The experimental determination of the CO_2-pressure in the venous blood is thus a matter of considerable interest, and we have made a number of experiments on the subject.

We first tried the method of holding the breath for a certain time after a normal expiration or inspiration, and endeavouring to deduce the venous CO_2-pressure from the rise in the percentage of CO_2 in the alveolar air. The alveolar CO_2-pressure continues to rise for as long a period as it is possible to hold the breath: there is no pause indicating the venous CO_2-pressure. The following series, of which each figure is an average of two or more observations, will illustrate this statement. The observations were made during rest, the breath being held at the end of either a normal inspiration or a normal expiration. Subject J. S. H.

Breath held after inspiration		Breath held after expiration	
	Alveolar CO_2 p.c.		Alveolar CO_2 p.c.
After 0″	5·57	After 0″	5·67
„ 10″	6·09	„ 10″	6·28
„ 20″	6·40	„ 20″	6·65
„ 30″	6·59		
„ 40″	6·90		

A volume of blood equal to the whole of that in the body passes through the lungs, as shown below, about once in 40 to 60 seconds in man during rest; but through some parts of the body the circulation time is doubtless much shorter than through others, so that even after 20 secs. some of the blood may have come round again, and thus raised the venous CO_2-tension abnormally, which would account for the continuous rise in the alveolar CO_2 percentage. It seemed to us, however, that the venous CO_2-pressure might be calculated from the rise of the alveolar CO_2-pressure during a short period—say 10 secs. Assuming that CO_2 is given off into the alveoli by diffusion, the rate of rise of alveolar CO_2-pressure when the breath is held for a given time must depend on (1) the difference in CO_2-pressure between the alveolar air and the venous blood, and (2) the volume of air in the lungs. If, now, we know the rate at which CO_2 is coming off with the normal difference of CO_2-pressure between venous blood and alveolar air, and the extent to which this rate is diminished as the alveolar CO_2-pressure rises, we can calculate the venous CO_2-pressure.

The volume of air in the lungs can easily be determined. We used a simplified form of the method introduced by Durig[1]. At the end of a normal expiration the subject inhaled from a small bag an accurately measured volume (about 1·25 litres) of pure oxygen from a small bag. By several rapid and deep exhalations and inhalations the oxygen was thoroughly mixed with the air of the lungs, and the nitrogen percentage finally determined. From the nitrogen percentage of the mixture it was of course easy to determine the volume of air in the lungs. That the method is accurate was shown as follows. From the volume at the end of a normal expiration, as found by this method, the residual air was calculated by making a maximal expiration through a gas-meter at the end of a normal expiration. The residual air was then determined directly by the oxygen method, and found to agree with the calculated value within a few c.c. Thus for C. G. D. the air (measured at ·15°) in the lungs was found on different days to amount to 3380 and 3150 c.c. at the end of a normal resting expiration; and the additional air which could be expired was 1855 and 1610 c.c., so that the calculated residual air was 1525 and 1540 c.c. The residual air as directly determined was 1565 and 1557 c.c.

The following are the results of an experiment on the venous CO_2-pressure of J. S. H.

The volume of air in the lungs at the end of a normal expiration was found to be 3360 c.c. (at 16°). The CO_2-production, as determined by the bag method of Douglas, was 3·84 c.c. per second (at 16°). The normal alveolar CO_2 percentage was 5·765 (mean of several inspiratory and expiratory samples); and the mean alveolar CO_2 percentage after holding the breath for 10 secs. was 6·34 p.c.—a rise of 0·575 p.c. At least 5 minutes of complete rest was allowed before each determination. Now it is clear that if CO_2 had continued to come off at a normal rate while the breath was held, $10 \times 3{\cdot}84 = 38{\cdot}4$ c.c. of CO_2 would have come off into the lungs, and this would have raised the alveolar CO_2 percentage by 1·14 p.c., while the actual rise was only about half of this, although the oxygen percentage was, to judge from other experiments, falling at about a normal rate. Hence the discharge of CO_2 was being diminished greatly by the rise of alveolar CO_2-pressure. The venous CO_2-pressure was calculated as follows[1]:

---

[1] Durig. *Cntrlb. f. Physiol.* XVII. p. 258. 1903.

Let p = difference of CO_2-pressure between normal alveolar air and venous blood (when oxygenated), and x = increase of alveolar CO_2-pressure after t secs. At a pressure-difference p, 3·84 c.c. of CO_2 per sec. were given off by the blood; and at a difference $p-x$, $\frac{3{\cdot}84\,(p-x)}{p}$, causing a rise in percentage of CO_2 in the 3360 c.c. of air in the lungs of

$$\frac{3{\cdot}84 \times 100\,(p-x)}{3360p} = \frac{4\,(p-x)}{35p}\ \text{°/}_\text{o}\ \text{per second},$$

$$\therefore\ \frac{dx}{dt} = \frac{4\,(p-x)}{35p},$$

$$\therefore\ t = \frac{35p}{4}\int_0^x \frac{dx}{p-x}$$

$$= \frac{35p}{4}\log_e\left(\frac{p}{p-x}\right).$$

Now t was $= 10$, and $x = 0{\cdot}575$ in the experiment; and if we insert these values and solve the equation, $p = 0{\cdot}725$ p.c. or 5·15 mm. The CO_2-pressure of the oxygenated venous blood would therefore be $5{\cdot}765 + {\cdot}725 = 6{\cdot}49$ °/o.

On reference to Fig. 3 it will be seen that a rise of 5·15 mm. of CO_2-pressure corresponds to a difference of only 2·35 vols. of CO_2 per 100 c.c. between venous and arterial blood. This difference must be calculated on the middle curve in Fig. 3, as the venous blood becomes oxygenated before it gives off all its CO_2. The result is suspiciously low. It would imply (since the respiratory quotient at the time was 0·75) that in passing through the body the blood lost only 17·3 p.c. of its oxygen, and was taking up in the lungs only 3·13 c.c. of oxygen (at 0°) per 100 c.c. of blood, while the lung ventilation was removing 4·46 c.c. per sec., or 268 c.c. per min. The circulation of blood would therefore be at the rate of 8·6 litres per minute—a higher rate than is probable from other estimates.

On re-considering the matter we came to the conclusion that there is a fallacy in the calculation: for as the pressure of CO_2 in the lung rises a certain amount of CO_2 must go into solution and combination in the lung tissue, and this is not allowed for in the calculation, nor are there any data for estimating the amount. The real venous CO_2 tension must therefore be higher than the estimate.

We therefore adopted another method, which consisted in applying the principle of the Pflüger aerotonometer, using the lungs themselves as the aerotonometer. After a maximal expiration a maximal breath was taken in from a bag or Krogh spirometer containing a mixture of CO_2 and air. This breath was held in the lungs for about 5 secs., and about a litre then expired through a tube, and an alveolar sample taken. After a further interval another alveolar sample was taken similarly; and sometimes a third sample. If in the latter sample or samples the CO_2-pressure was lower or higher than in the first, we concluded that the venous CO_2-pressure was lower or higher than the pressure in the samples. We could thus infer the venous CO_2-pressure in the same manner as with the Pflüger aerotonometer.

---

[1] We owe the calculation to Mr **J. B. S. Haldane**.

The first experiments were made by J. C. Three alveolar samples were taken, after about 5, 9, and 13 secs. respectively. The following table shows the results of a series at different times by J. C. on herself.

TABLE V. *Percentages of CO_2.*

No.	In spiro-meter	In first alveolar sample	In second alveolar sample	In third alveolar sample	Venous CO_2-pressure (uncorrected for oxygenation)
1	7·2	6·22	6·90	7·04	>7·04
2	4·9	4·95	5·90	6·38	>6·38
3	8·60	7·30	7·14	7·22	7·2
4	8·30	7·33	7·26	7·24	7·25
5	14·2	—	8·34	7·75	<7·75
6	9·61	7·30	7·23	7·02	<7·02
7	7·98	6·56	6·67	6·82	>6·82
8	9·91	7·30	7·31	7·28	7·28
9	11·60	8·18	7·67	7·47	<7·47
10	6·83	6·39	6·67	—	>6·67
11	15·80	10·32	9·52	9·08	<9·08
12	9·0	7·71	7·56	7·53	<7·53
13	8·43	6·92	6·81	—	<6·81
14	6·85	6·52	—	6·81	>6·81
15	9·01	7·28	7·47	7·56	>7·56
16	10·3	7·83	7·68	7·65	<7·65
17	10·04	7·90	7·66	7·66	7·66
18	8·12	6·89	6·74	6·78	6·76
19	8·56	7·00	7·14	7·18	>7·18
20	10·37	7·67	7·57	7·50	<7·50
21	9·50	7·31	7·14	7·04	<7·04
22	8·00	6·87	6·71	6·70	6·70
23	8·20	—	6·63	6·69	>6·69
24	8·15	6·55	—	6·34	<6·34
25	7·73	6·79	6·77	6·87	?
26	8·48	6·83	6·62	6·50	<6·50
27	7·68	6·38	—	6·51	>6·50
28	6·63	6·08	6·30	6·40	>6·4
29	6·84	6·10	6·34	6·30	6·3
30	8·05	6·73	6·77	6·68	6·7

In exps. 1 to 8 the spirometer contained oxygen and CO_2: in the others air and CO_2. It will be seen that the oxygen experiments gave from 7·0 to 7·3, while the air experiments up to No. 20, gave from 6·76 to 7·66. Up to No. 20 no special care had been taken about resting before each experiment; and the period of rest, sitting, was usually only about half to one minute, and somewhat variable. From No. 21 onwards a period of ten minutes' rest was allowed before each experiment, with the result, as will be seen, that lower and steadier results were obtained, varying between 6·3 and 6·7, with a mean of about 6·5. The normal resting alveolar CO_2 percentage in J. C. averaged about 4·90.

Four observations were made on another lady (H. C.), after 10 minutes' rest in each case. The results were $< 6·60$, $< 6·44$, $> 6·06$, $> 6·08$. The result indicated was about 6·25 °/₀. The value obtained for the normal alveolar CO_2 percentage was 4·75 °/₀. Hence the results for both venous and arterial CO_2-pressure were slightly below those for J. C.

In a further series on J. S. H., C. G. D., and Dr J. G. Priestley only two alveolar samples were taken—one after 4½″ and one after 10½″ from the end of the deep inspiration—from a bag containing a mixture of CO_2 and air. The results were as follows:

TABLE VI.

		Percentages of CO_2				
Subject	Number	In bag	In first alveolar sample	In second alveolar sample	Venous CO_2-pressure (uncorrected for oxygenation)	Normal alveolar air
J. S. H.	1	8·48	7·22	6·99	—	—
,,	2	6·96	6·61	6·80	6·9	5·87
C. G. D.	3	8·48	7·39	7·22	—	—
,,	4	6·96	6·72	6·97	7·1	5·60
J. S. H.	5	8·98	7·24	7·08	—	—
,,	6	6·93	6·70	6·91	7·0	5·83
J. G. P.	7	8·98	7·51	7·55	7·55	—
,,	8	6·93	6·88	6·83	6·8	—
,,	9	6·93	6·79	6·80	6·8	5·70
J. S. H.	10	9·27	7·25	7·03	—	—
,,	11	6·77	6·80	6·87	6·9	5·73
C. G. D.	12	9·27	7·80	7·30	—	—
,,	13	6·77	6·71	6·71	6·7	5·60
J. G. P.	14	9·27	7·79	7·53	—	—
,,	15	6·77	6·41	6·49	6·7 ?	—

In the first seven of these experiments the subjects had not been careful to rest thoroughly before each determination. In No. 7, for instance, which gave an abnormally high result, the subject had only rested about a minute. In No.'s 8 and 9, made shortly afterwards, he rested for ten minutes before each experiment, and the results were a good deal lower. In the rest of the experiments the same precaution was taken.

It will be seen that in all the subjects the values obtained for the uncorrected venous CO_2-pressures during complete rest (in a sitting position) came to from 6·3 to 6·9 p.c. These values, however, are for venous blood after oxygenation in the lungs, and hence do not represent true venous CO_2-pressures. To obtain true values we must allow for the influence of oxygenation. This, as will be seen from Fig. 3, reduces by about 40 p.c. the differences between arterial and venous CO_2-pressure. Thus in the case of J. S. H. the uncorrected difference between arterial and venous CO_2-pressure was $6·9 - 5·73 = 1·17$ p.c. Hence the real difference was only 0·70 p.c., and the true venous CO_2-pressure was $5·73 + 0·70 = 6·43$ p.c. or (allowing for aqueous vapour and existing barometric pressure) 45·6 mm., as compared with 40·6 mm. in the arterial blood. In the four subjects investigated the values obtained for rest were as follows:

Subject	Arterial CO_2-pressure	Venous CO_2-pressure	Difference
J. C.	34·9	41·8	6·9
J. S. H.	40·6	45·6	5·0
C. G. D.	39·7	44·4	4·7
J. G. P.	40·4	45·1	4·7
Mean	38·9	44·2	5·3

The venous CO_2-pressures for J. S. H. and C. G. D. are about 1·5 mm. higher than those given by the method of calculating from the composition of the alveolar air after holding the breath for 10 seconds, and this lack of correspondence is presumably due to the source of error already referred to. The average difference between arterial and venous CO_2-pressure is nevertheless still remarkably small. Reference to Fig. 3 shows that the venous blood had only taken up about 24 p.c. of the CO_2 which it would take up if all its oxygen were used up. Hence the blood had only lost about 24 p.c. of its oxygen in passing round the circulation; and in C.G.D., J.S.H., and J.G.P. the proportion lost was only from 21 to 22 p.c., though it was 31 p.c. in J. C. As the total available oxygen of the blood is about 18 c.c. per 100 c.c. of blood, 24 p.c. of this would be 4·3 c.c. Taking the resting metabolism at 300 c.c. of oxygen per minute, this gives an average blood-circulation of 6·7 litres per minute, or about 7·7 litres in three of the subjects and 5·0 in the other. The blood-volume in J. S. H. and C. G. D. is about 4·7 litres. Hence in them a volume of blood equal to the whole of that in the body was passing round once in about 37 seconds on an average.

The mean rate of circulation, and percentage saturation of the venous blood with oxygen, are high as compared with other estimates—for instance those of Krogh and Lindhard[1] by their nitrous oxide method. We cannot, however, see any reason for suspecting that our results are too high. It appears that the circulation does not increase in direct proportion to the metabolism. The difference between complete and incomplete rest seems to make a considerable difference in the venous CO_2-pressure, as has already been shown; and during any exertion the difference between arterial and venous CO_2-pressure appears to increase very greatly. For instance we observed that on holding the breath for six seconds during exertion the alveolar CO_2-pressure rose by 20 mm., which indicated a very great increase in the difference between arterial and venous CO_2-pressure.

It is evident that our method of determining venous CO_2-pressure may be applied also to the determination of venous oxygen pressure, as well as to direct determinations of venous CO_2-pressure, without any correction for the effects of oxygenation of the blood in the lungs. All that is necessary is that such a mixture of nitrogen or hydrogen, with CO_2 and oxygen, should be inhaled that neither the percentage of oxygen nor that of CO_2 shall alter appreciably between the two or three alveolar samples taken for analysis. We have made a number of experiments by this method, and, so far, they confirm the conclusion that the difference, during rest, between the arterial and venous pressures of CO_2 and oxygen is a good deal less than has hitherto been supposed—in other words that the resting blood-circulation is unexpectedly rapid. As, however, we wish to extend our experiments in several directions, we have decided to leave this subject for a further paper.

Our results for venous CO_2-pressure seem to harmonise well with those obtained in man with the lung-catheter method by Loewy and v. Schrötter[2]. These authors blocked one lung by means of a lung-catheter, and used it as an aerotonometer for determining the gas-pressures of the venous blood. By this method they found that the average venous CO_2-pressure was 42·5 mm., with an oxygen-pressure of

---

[1] *Skand. Arch. f. Physiol.* XXVII. p. 100. 1912.

[2] Loewy and v. Schrötter. *Untersuchungen über die Blutcirculation beim Menschen*, Berlin, 1905.

37·5 mm. The oxygen-pressure must have been abnormally low, as half of the blood was not being oxygenated, and the other lung cannot compensate for the deficiency in oxygenation, although it can, and doubtless did, compensate for the deficiency in elimination of CO_2. Our own results give a higher figure for the venous oxygen-pressure. The lower oxygen-pressure in the arterial blood would doubtless lead to a slight increase in the breathing; and allowing for this, Loewy and v. Schrötter's results seem to agree very closely with our own.

SUMMARY[1].

(1) Under normal conditions the curve representing the relation between pressure of CO_2 and amount of CO_2 taken up by fresh defibrinated human blood is very definite and constant for the same individual, and does not differ much for different individuals. At 40 mm. pressure of CO_2 100 volumes of oxygenated human blood take up about 50 volumes of CO_2; and at about 80 mm. pressure 15 additional volumes are taken up.

(2) At a given pressure of CO_2, blood which has been deprived of oxygen takes up considerably more CO_2 than oxygenated blood. The oxygen (and the same is true for CO) thus tends to drive out CO_2, and this action depends on the saturation of the hæmoglobin. A curve is given, showing the extent of this action with varying pressures of CO_2.

(3) The oxygenation of the blood in the lungs helps to drive out CO_2, and increases by about 50 p.c. or slightly more the amount of CO_2 given off at each round of the circulation. The de-oxygenation of the blood in the tissues helps the absorption of CO_2 and diminishes by about 40 p.c. the rise of CO_2-pressure and of hydrogen ion concentration in the venous blood.

(4) It follows from (3) that the giving off of CO_2 by the lungs is to a large extent consequent on the taking up of oxygen, and that under conditions where the uptake of oxygen is due to the physiological activity of the lung epithelium the giving off of CO_2 is also dependent on this activity.

(5) With the help of the curves referred to under (1) and (2), and observations of the rise or fall of alveolar CO_2-pressure when the breath is held after inhaling mixtures of CO_2 and air, it is possible to calculate the CO_2-pressure of the venous blood reaching the lungs, and the rate

---

[1] A short abstract of our main conclusions appeared in the *Proceedings of the Physiological Society*, This *Journal*, XLVII. p. ii. 1913.

of blood-flow through the lungs. Measurements made during rest gave an average venous CO_2-pressure of 44·2 mm., and a rate of blood-flow of 7·7 litres per minute was calculated for a person with a total blood-volume of 4·7 litres.

In a paper which appeared while the present paper was in the press Morawitz and Walker (*Biochem. Ztschr.* LX. p. 395, 1914) describe a method of using the CO_2 absorbed by blood in presence of a given pressure of CO_2 and temperature as an index of the reaction of the blood. Their observations with this method including experiments on muscular work, are in complete harmony with those contained in the first part of our paper, and we are in agreement with the main conclusions which they draw.

Part of the apparatus used in our experiments was obtained from a grant from the Government Grant Committee of the Royal Society.

THE INFLUENCE OF DISTENTION OF THE LUNGS ON HUMAN RESPIRATION. BY JOHANNE CHRISTIANSEN AND J. S. HALDANE.

(*From the Physiological Laboratory, Oxford.*)

As is well known, **Hering** and **Breuer** were the first to describe the long pause in respiration on distention of the lungs with air, and to show that this pause is absent after section of the vagi. They also found that the pause still occurs when hydrogen is used for distending the lungs, and rightly concluded that the pause depends on vagus excitation due to stretching of the lungs, and not on chemical changes in the blood. Their experiments were made with dogs, and many experiments relating to the same phenomenon have been made with rabbits, but hitherto none seem to have been made on man.

For distending the lungs we used a bag containing air, and so weighted that the air was under a pressure of 6 to 8 cm. of water. The subject sat in a perfectly easy reclining position, and breathed through a wide-bored three-way tap, of which one branch led to the bag, another to the air of the room, and the third to the mouthpiece. The nose was closed by a clip. The subject at first breathed quietly for a short time through the branch of the tap connected with the air of the room; and at the end of an expiration the tap was suddenly turned so as to connect the mouthpiece with the air under pressure in the bag. The consequence of this was that the lungs were rapidly distended with a large volume of air. For recording the respirations we used a stethograph connected with one of the recording tambours of a Mackenzie polygraph. The subject was careful to remain passive during the experiment, and refrain from all voluntary resistance to the distention or interference with the breathing.

The invariable result of this experiment was that the respiratory movements ceased entirely, for usually about half a minute. The pause was then broken by a deep expiration, followed by a further pause before the next expiration, and so on with increasingly shorter pauses.

On turning the tap so that the subject again breathed atmospheric air the pauses disappeared, and the breathing returned to normal, although it was deeper than usual for a few breaths. A tracing of these effects is shown in Fig. 1. The pause is similar to that observed in dogs or chloralised rabbits with intact vagi; but in rabbits the pause is broken by an inspiratory effort, as was first pointed out by Guttmann[1], whereas in the dog and in man an expiratory effort breaks the pause.

When the lungs are distended with air the alveolar CO_2-percentage is of course lowered considerably for the time; and the consequent lowering in the CO_2-pressure of the arterial blood must have some influence on the respiratory centre. That the pause is not due merely to lowering of the alveolar CO_2-pressure was shown by Haldane and Lorrain Smith[2] for the rabbit. They found that a pause is produced even when the lung is distended with air containing a high percentage of CO_2, and during actual hyperpnœa from CO_2. Fig. 2 shows a tracing for the same subject as in Fig. 1, but with the air in the bag containing 7·3 °/$_0$ of CO_2 and 8·2 °/$_0$ of O_2. It will be seen that the pause is produced, just as with pure air; but that the pauses succeeding the first diminished in length much more rapidly than when pure air was used, and that on releasing the pressure, and breathing pure air again, there was marked hyperpnœa for some time. The effects in man are similar in essential details to those in the rabbit.

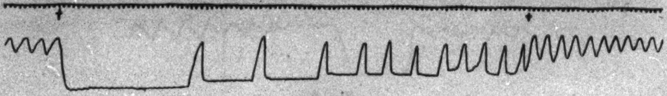


Fig. 1.

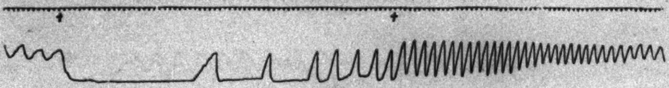


Fig. 2.

Figs. 1 and 2. Effects of prolonged distention of the lungs. To be read from left to right. Time-marker=seconds. Distention continued between the two crosses. In Fig. 1 pure air was used for distention: in Fig. 2 air containing 7·3 °/$_0$ of CO_2 and 8·2 °/$_0$ of oxygen.

Although the Hering-Breuer inhibitory effect is not dependent on chemical changes in the blood we have obtained clear evidence that the chemical changes will not merely break through the inhibitory effect when there is increase of CO_2 in the alveolar air, as in the last experiments, but will augment it when the alveolar CO_2 is diluted with pure air. We found that when the distention with pure air was only continued for a few seconds, and the pressure then released, the apnœic pause continued for some time afterwards, as shown in Fig. 3. This result might at first sight be attributed to the Hering-Breuer distention effect lasting over the actual period of distention, and so producing a true apnœa in the manner generally assumed before the work of Haldane and Priestley. We found, however, that when the bag was filled with air containing sufficient CO_2 to prevent any appreciable fall in the alveolar CO_2-percentage there was no apnœa following a short distention. 4·6 % of CO_2 in the bag was sufficient for this purpose. Fig. 4 shows a tracing, with the same subject as in Fig. 3.

---

[1] *Arch. f. Anat. u. Physiol.* p. 100. 1875.

[2] *Journ. of Pathol. and Bact.* I. p. 168. 1892.

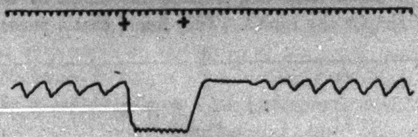


Fig. 3.

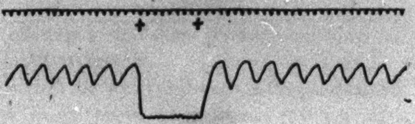


Fig. 4.

Figs. 3 and 4. Effects of distention for 8 secs. Crosses show beginning and end of distention. To read from left to right. In Fig. 3 pure air used for distention: in Fig. 4 air containing 4·62 % CO_2.

By very short exposures to the distention effect it was possible to distinguish quite clearly between the Hering-Breuer inhibition and the chemical apnœa. Figs. 5, 6, and 7 show the effects of periods of full distention of about 1, 3, and 5 seconds. It will be seen that with only 1 to 3 seconds' distention the breathing returns at once after the distention, but dies away again after a few seconds, which is succeeded by an apnœa of a few seconds' duration. With five to ten seconds' full distention, on the other hand, the apnœa follows immediately on the release of the distention. Similar results were obtained on another subject. It is clear that if the release of pressure is so timed that the block of "apnœic" blood resulting from the lowered alveolar CO$_2$-percentage has time to reach and act on the respiratory centre, a chemical apnœa prolongs the Hering-Breuer apnœa. If, however (as in Fig. 1), the distention is continued for so long that the alveolar CO$_2$-percentage has time to rise to or above normal, and the corresponding blood to reach and act on the centre, there is no chemical apnœa; and chemical apnœa is also absent if the alveolar CO$_2$-percentage is, by adding CO$_2$ to the air in the bag, prevented from falling (Fig. 4).

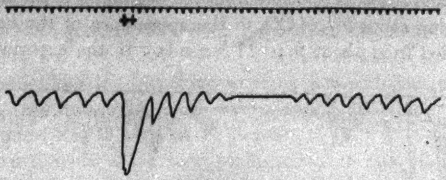


Fig. 5.

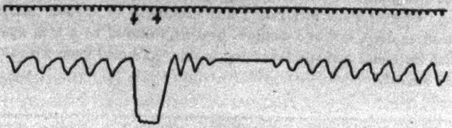


Fig. 6.

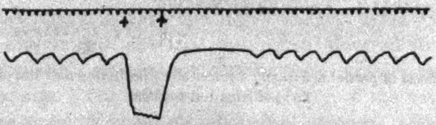


Fig. 7.

Figs. 5, 6, and 7. Effects of distention with pure air for increasing short periods. Crosses show beginning and end of distention. To read from left to right. Fig. 5 distention for 1 sec.: Fig. 6 for 3 secs.: and Fig. 7 for 5 secs.

It is evident that if we can thus trace a block of "apnœic" blood through the respiratory centre, it ought to be possible to trace a block of "hyperpnœic" blood also. The following experiment (which was repeated on various individuals) shows that this can easily be done. An apnœa which would normally last for about a minute was produced in the ordinary way by forced breathing during one minute. A few seconds after the beginning of the apnœa a deep breath was taken of air containing a high percentage of CO_2, and held for about 2 or 3 seconds. After this the apnœa continued at first for several seconds, but was then broken by two or three deep breaths. These then died away, and the apnœa continued till about the ordinary period of a minute from the cessation of the forced breathing. Fig. 8 shows the tracing from an experiment in which a breath of air containing 9·0 % of CO_2 was inhaled. In a corresponding experiment where a deep breath of air was taken, in place of the air containing CO_2, there was no break in the succeeding apnœa before it finally ceased. In different experiments and subjects the time from the beginning of the inhalation of air containing about 9 % of CO_2 to the appearance of the break in the apnœa varied from about 9 to 11 seconds. In the experiments where

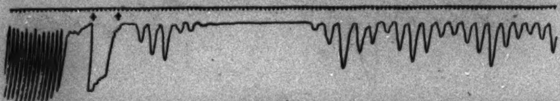


Fig. 8. Effect of a breath of air containing 9·0 % of CO_2 during apnœa following forced breathing. Crosses show inspiration and expiration of breath. After an interval there are three deep, and two shallow, breaths, followed by a long apnœic interval, after which the usual periodic breathing begins. To read from left to right. Time-marker = 1 second.

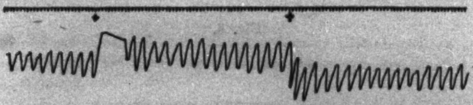


Fig. 9. Effects of partial deflation. Crosses show beginning and end of deflation. To read from left to right.

chemical apnœa followed distention of the lungs with pure air the interval between the beginning of the distention and the apnœa was usually less—as little as 6 to 8 seconds in J. S. H., and about 8–14 seconds in C. G. D. The interval depended on the depth of the distention and the time during which it was continued. This is what might be expected with a chemical apnœa, since the effect must differ according to the difference between the threshold exciting percentage of CO_2 and the actual alveolar CO_2 percentage during the distention: also, within certain limits, on the time during which this difference is maintained. The time required for the blood to pass from the lungs to the respiratory centre, and to affect the latter, was thus as little as 6 to 8 secs.; and the time actually required for the blood to pass from the lungs to the capillaries of the centre was probably a good deal less. The prompt action of the blood on the centre is easily intelligible, since it was shown by Campbell, Douglas, Haldane and Hobson[1] that apnœa is produced by a diminution of as little as 0·2 % in the alveolar CO_2-percentage.

We have also tried the effects of partial deflation of the lungs. The partial deflation was produced by turning the three-way tap into connection with a vessel containing air kept at a negative pressure of as much as 15 cm. of water. Somewhat to our surprise the partial deflation did not give rise to a deep inspiration. Sometimes there was a short pause, due, apparently, to the mechanical hindrance to inspiration, but sometimes the breathing went on just as before, though with the lungs less full. Fig. 9 shows a tracing in which the partial deflation was followed by a short pause.

Our experiments afford new confirmation to the view that true apnœa, apart from the inhibitory nervous effects produced during actual distention of the lungs, or actual excitation along certain other nerve paths connected with the centre, is a "chemical" apnœa. This view was recently questioned by Foa[2], who repeated Fredericq's well known crossed circulation experiments, and obtained apnœa in both animals—a result which seemed to support the old view that distention of the lung leaves a nervous after-effect causing apnœa. The new experiments made by Leroy[3] in Fredericq's laboratory seem to render it practically certain that the crossing of the circulation was incomplete in Foa's experiments, and that the apnœa observed by Foa in his crossed circulation experiments was in both animals nothing but a chemical apnœa. The inherent improbability of the vagus theory of true apnœa was very great, on teleological grounds; and so far as we are aware there is now left no experimental evidence which can be used to support it.

We are indebted to Dr Douglas, and also to Professor Yandell Henderson who was in Oxford at the time, for much help with some of the experiments.

---

[1] This *Journal*, XLVI. p. 301. 1913.

[2] *Arch. di Fisiol.* VII. p. 195. 1909.

[3] *Arch. internat. de Physiol.* XIII. p. 322. 1913.

THE HEART, LUNG, KIDNEY PREPARATION.

BY F. A. BAINBRIDGE AND C. L. EVANS.

(*From the Institute of Physiology, University College, London.*)

THE methods hitherto generally employed for the perfusion of isolated mammalian organs are attended by certain drawbacks among which may be mentioned first the difficulty of ensuring adequate oxygenation of the blood and removal of carbonic acid, and secondly the absence of anything corresponding to the pulsatile flow of blood normally produced by the heart. In the case of some organs these drawbacks are not of great importance. The kidneys however are so sensitive to lack of oxygen, and probably also to the absence of the intermittent driving force of the heart, that perfusion of the isolated kidney has not yielded results which could justly be compared with those obtained in the living animal.

The heart-lung preparation devised by Knowlton and Starling(3) seemed to offer a means whereby these drawbacks might be obviated, and the kidneys might be perfused either *in situ*, or after removal from the body under conditions closely approximating to the normal; and the present paper deals mainly with the method employed to carry out such perfusions. The method may be described as a heart-lung-kidney preparation.

Methods.

Two dogs are used for each experiment—one for the heart-lung preparation and one for the kidney or kidneys. After a preliminary dose of morphia the dogs are anaesthetised with chloroform and ether. The heart-lung preparation is made from one dog according to the method of Knowlton and Starling(3). Simultaneously the kidney preparation is made on the other dog by one or other of the two following methods, and finally the heart-lung and the kidney preparations are connected.

(1) By this method the kidneys are left *in situ* and either one or both may be used for the perfusion experiment. After the removal of

the greater part of the small and large intestines the aorta and vena cava are tied off a short distance below the origin of the renal vessels. The suprarenal veins are next tied off and the suprarenal glands are put out of circulation so far as possible by tying ligatures round them. Ligatures are then placed round the aorta and the vena cava just above the origin of the renal vessels but they are not tied. The next and most important step consists in tying off all the vessels arising from the aorta and vena cava between the upper and lower ligatures just mentioned except the renal arteries and veins. This is best effected by raising the left kidney from its bed so as to expose the posterior aspect of the aorta and vena cava from which arise many small lumbar vessels, one at least being quite close to the origin of the renal arteries. Any considerable leakage will interfere with the success of the subsequent experiment, and it is essential to occlude these vessels as completely as possible. If this part of the operation involves much handling of the left kidney it is desirable to ligature it off and to use only the right kidney for the experiment.

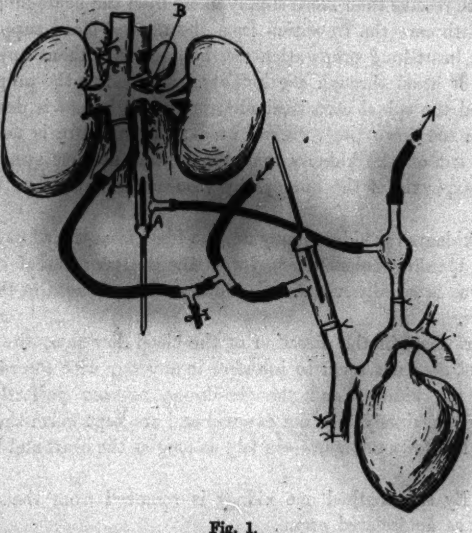


Fig. 1.

The aorta and vena cava are then clamped just below the renal vessels and cannulæ are placed in them below the clamps and are connected with the heart-lung preparation as shown in Fig. 1. The cannulæ contain a little hirudin solution and a thermometer is placed in the arterial cannula *A*. Up to this point of course the kidneys are receiving their blood supply by the normal route.

Finally the cannula *A* is filled with blood from the heart-lung preparation, the clips on the aorta and vena cava are removed, and the blood is allowed to pass upwards along the aorta through the kidneys. At the same moment the ligatures *B* and *C*, previously passed round the aorta and vena cava, above the renal vessels are tied off, and the kidneys henceforth receive their blood supply solely from the heart-lung preparation. With care the transition from the normal blood supply to that from the heart-lung preparation can be effected without any pause in the flow of blood through the kidneys. As soon as the preparation is completed the spinal cord is divided in the neck, and is destroyed by passing a rod down the vertebral canal; this measure is necessary in order to cut off the kidneys from the central nervous system and to prevent asphyxia of the kidneys by vaso-constriction when the dog is killed.

The kidneys (or kidney) may now be freed from their fatty covering and any bleeding points touched with the cautery; loss of blood from this source is usually trivial. Cannulæ are then placed in the bladder or ureters.

The reversal in the direction of the blood flow along the aorta and vena cava does not seem to interfere in any way with the rate of flow through the kidneys and the circulation appears perfectly normal. Provided the kidneys are not exposed and are kept warm the preparation will last for some hours—in fact as long as the heart and lungs hold out.

(2) By this method one kidney is removed from the body and perfused as an isolated organ.

After the removal of the greater part of the small and large intestines a ligature is passed round one renal vein close to its junction with the vena cava and is left loose. The kidney on that side is gently raised from its bed, the renal artery is exposed for $\frac{1}{2}$–$\frac{3}{4}$ of an inch and a ligature is passed round it. As soon as the heart-lung preparation is ready the cannula *A* (Fig. 2) which is to be inserted into the renal artery is filled with hirudinized blood and a screw clamp *B* is so adjusted that a slow but steady stream of blood is flowing from the cannula.

The renal artery is then occluded by a clip near its origin, the artery is opened beyond the clip and the cannula is at once inserted and tied in the artery. At the same moment the other operator ties off the renal vein close to the vena cava. Immediately the ligatures are tied, the renal vein is divided to allow the blood to escape and the screw clamp *B* on the tube conveying arterial blood from the heart-lung preparation is loosened. The kidney is rapidly removed from the body, placed in a funnel and supported on gauze. The blood escaping from the renal vein passes down the funnel and into the reservoir *C*. The funnel containing the kidney is covered by a piece of oiled silk to prevent evaporation. A cannula may be placed at leisure in the ureter. The general arrangement of the complete preparation is shown in Fig. 2.

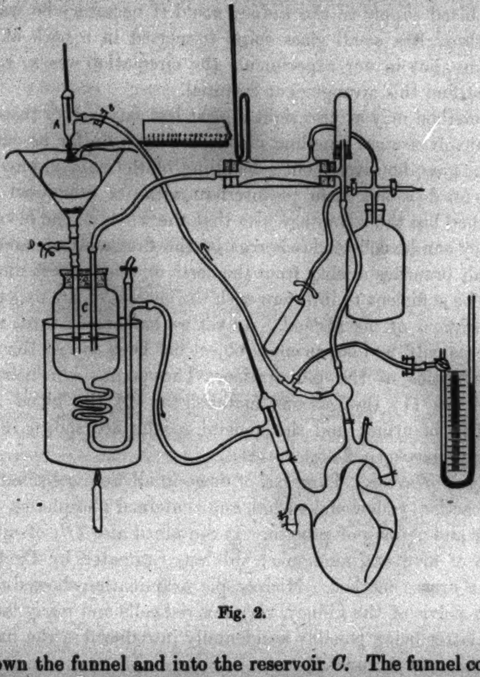


Fig. 2.

It has been found that the transition from the normal circulation to that provided by the heart-lung preparation occupies about a minute including the tying of the ligatures, but the period during which the kidney is deprived of arterial blood is less than ½ minute since the arterial blood enters the kidney directly the cannula is placed in it, and before it is actually tied in.

In order to avoid delay in inserting the cannula into the renal artery and establishing the new circulation it is desirable to ascertain beforehand that the arterial cannula is of suitable size. The rate of flow of blood through the kidney is determined by opening the clip *D* on the side tube of the funnel and pinching the rubber connection below it. The blood supply to the kidney could if necessary be warmed by passage through a small glass spiral immersed in a bath at suitable temperature, but in our experiments the circulation was so rapid that we did not find this arrangement essential.

This method may at first sight appear less satisfactory than the first since it involves more handling of the kidney. So far as our limited experience goes, however, gentle handling of the kidney, provided it is kept warm, does not seem to interfere with its functional capacity. This method has the advantage also that the whole of the blood leaving the kidney can be collected, whereas by the first method some leakage from small branches arising from the aorta or vena cava is unavoidable and may be sufficient to interfere with the success of the experiment.

Applications of the Method. As yet we have made three successful experiments only, and our primary object has been to test the efficiency and possibilities of the preparation. The points which have been examined are (1) the gaseous metabolism, (2) the blood flow, and (3) the flow of urine; and the general results are shown in the two following protocols (p. 283).

Analysis of urine. Three c.c. of urine in all were collected. It was acid in reaction, yellow and turbid, and contained phosphates, chlorides, sulphates and a trace of protein. It contained also 1 °/₀ of nitrogen in the form of urea and ammonia: this was estimated by Dr Plimmer using the urease method. Microscopic examination showed some cells from the pelvis of the kidney, very few red cells and many fat droplets (the two latter being possibly accidentally introduced in the insertion of the cannula into the ureter).

Gaseous metabolism. The gaseous metabolism was determined in successive periods of 20 minutes by the method employed by Evans(4) for the study of the metabolism of the heart-lung preparation. In one experiment the metabolism of the heart-lung preparation alone was first determined, and then that of the same preparation with the

I. The preparation was made by the first method, the kidneys being kept *in situ*. One kidney was used. Heart-lung preparation ready at 2.30, and the preparation completed at 3.40.

Weight of heart=37 grms.: weight of kidneys=30·5 grms.

Temperature of blood entering heart was 36·5° C.: pulse rate 154 per minute. Output of heart=395 c.c. per minute. Blood-pressure remained constant at 100 mm. Hg. Temperature of blood entering kidneys=34–35·5° C. No urine secreted.

Gaseous metabolism of heart-lung preparation was first determined, and subsequently that of heart-lung-kidney preparation: the figures for the metabolism of the kidney are determined by difference. The observations were made by Evans' method(4).

Preparation	Time	Total c.c. O_2 used per hour	Total c.c. CO_2 given off per hour	O_2 used per hour by kidney c.c.	O_2 used per 1 grm. of kidney hourly c.c.	CO_2 given off per hour by kidney c.c.	Respiratory quotient	Blood-flow through kidney in c.c. per 1 min.
Heart-lung	3.0 —3.20	200·1	147	—	—	—	0·735	—
„	3.20—3.40	188·0	136·5	—	—	—	0·745	—
Heart-lung-kidney	3.40—4.0	291·9	193·5	95·4	3·13	51·5	0·662	Not observed
„	4.0 —4.20	282	197·4	85·5	2·81	55·4	0·70	29
„	4.20—4.40	288	219	91·5	3·0	77·0	0·76	35
„	4.40—5.0	280·5	201	84·0	2·76	59·0	0·71	12·5
„	5.0 —5.10	285	216	88·5	2·9	74	0·755	55·0

The rate of the blood-flow through the kidneys was varied by means of a screw clamp on the arterial tube to the kidney.

Mean respiratory quotient of kidney=0·71.

Average O_2 consumption by kidney=0·046 c.c. per 1 grm. per minute.

II. The preparation was made by the second method, and one kidney was used.

Weight of heart=79 grms.: weight of kidney=35·5 grm.

Only oxygen consumption was measured, and œdema of the lungs prevented any direct observation at the end of the experiment of the oxygen used by the heart. The oxygen consumption of the heart and lungs was calculated from previous figures (obtained by Evans) at 3·08 c.c. O_2 per 1 grm. per hour.

Preparation	Time	Remarks	Urine movement in cms. along scale	Total O_2 used per hour c.c.	Calc. O_2 used by kidney per hour c.c.	O_2 used by kidney per 1 grm. per hour c.c.	Rate of blood-flow in kidney in c.c. per 1 min.
Heart-lung-kidney	4.53—5.13	Normal	0	393	85	2·4	80—90
„	5.13—5.33	*5.15* add to blood 2 c.c. 50 °/₀ $NaSO_4$	14	432	124	3·5	80—98
„	5.33—5.53	*5.28* 1 c.c. $NaSO_4$	33	462	154	4·34	80—84

The urine flowed along a tube provided with a scale graduated in centimetres. It flowed along the tube in spurts synchronous with visible contractions of the pelvis of the kidney and the ureter; these contractions took place at the rate of about 12 per minute.

addition of the kidneys, the metabolism of the kidney being therefore estimated by the difference. The oxygen consumption of the kidneys in these two experiments during the period when no urine was being secreted was fairly constant; the average consumption per grm. of kidney per minute being 0·046 c.c. in the first, and 0·040 c.c. in the second experiment. These figures are quite comparable with those obtained by Barcroft and Brodie (1) on the dog, which varied from 0·008 to 0·075 c.c. and with those of Barcroft and Straub (3) which varied from 0·03 c.c. to 0·1 c.c.; in both these sets of experiments some urine was being formed by the kidneys.

In the first experiment the effect of varying the rate of blood flow through the kidney upon the consumption of oxygen was investigated. It will be seen that with a flow varying from 12½–55 c.c. per minute the oxygen consumption over a period of 20 minutes showed very little variation. This result confirms the observation of Barcroft and Müller (2) on the submaxillary gland that the oxygen consumption of a tissue is determined primarily by the needs of the tissue and not by the blood supply, provided that the latter reaches or exceeds a certain necessary minimum.

In the second experiment in which the addition of sodium sulphate evoked an immediate flow of urine the consumption of oxygen reached 0·07 c.c. per grm. per minute, a figure quite comparable with those obtained by Barcroft and Brodie, and by Barcroft and Straub with the same diuretic. In one of Barcroft and Brodie's (1) experiments the injection of sodium sulphate increased the oxygen consumption from 0·015 to 0·074 c.c. per 1 grm. per 1 minute.

Unfortunately, in another experiment in which urine was obtained when the blood was diluted with Ringer's solution the oxygen consumption of the kidneys was not determined. The output of carbonic acid and the respiratory quotient varied considerably in successive periods of 20 minutes, these variations being no doubt due to different rates of washing out of CO_2 by the circulating blood, but the mean quotient in Exp. 1 is not very different from that of the heart, and is probably fairly accurate. Since the figures for the oxygen consumption of the kidneys are obtained indirectly, their accuracy will be affected by definite alterations in the work and consequently the gaseous metabolism of the heart, and it is necessary to guard against this source of error. Evans has shown, however (4, 6), that the average oxygen consumption of the heart-lung preparation apart from the presence of adrenalin, or violent changes of blood-pressure, is fairly constant; and the accuracy of our method could easily be checked by direct observation of the oxygen consumption made simultaneously with Barcroft's apparatus. In the two experiments just described these sources of error could be practically disregarded, and the results show that the gaseous metabolism of the kidneys in the heart-lung-kidney preparation is similar in amount to that observed by other methods for the kidneys in the body.

The blood flow. The amount of blood flowing through the kidneys can be varied at will, and depends upon the relative resistance of the renal vessels, and of the artificial resistance introduced into the heart-lung preparation. In our experiments, this resistance amounted to about 60–70 mm. of Hg., and the general arterial pressure was about 100 mm. Hg. By tightening a screw clamp on the tube supplying blood to the kidneys the rate of flow through the kidneys could be diminished at will. In the second experiment in which the pressures were those just mentioned the rate of blood flow through the kidneys was 80–98 c.c. per min. In Barcroft and Straub's (3) experiments the flow varied from 50–85 c.c. per min. in the absence of any diuretic. In our experiments owing to the loss of vasomotor control the renal vessels would possess little or no tone.

The flow of urine. The flow of urine may be regarded as the fundamental test of the success of the preparation. In two of our experiments urine was readily obtained, in one case after dilution of the blood with Ringer's solution, and in the other after the addition of sodium sulphate to the blood. In none of the three experiments was a spontaneous flow of urine obtained as soon as the preparation was started. We were at first inclined to attribute the absence of urine at the outset of the experiment either to the effect of the anæsthetic on the kidney, or to the experimental procedure itself. It must be remembered, however, that in these experiments the blood is rather concentrated, and that it remains practically constant in composition since it does not receive urea or other tissue products. It seems possible therefore that the kidney does not form urine simply because the blood is not providing an adequate chemical stimulus. The readiness with which urine is formed when the blood is diluted, or on the addition of a diuretic, favours this view, but further investigations will be made.

The action of sodium sulphate (protocol II) was interesting in that it evoked a prolonged flow of urine without appreciably altering either the concentration of the blood or the rate of flow through the kidney.

It did, however, notably increase the gaseous exchange, and the analysis of the urine showed that the latter was a true secretion.

On the whole our observations entirely confirm and agree with those obtained by other workers using different methods, and show that the heart-lung-kidney preparation will yield results comparable with those obtained in the living animal. It seems fair to conclude then that the preparation is a reliable one and that the kidneys are in a normal functional condition. The advantages of the preparation are, first, that the blood-pressure and the rate of blood flow through the kidney are under control, and can be varied at will, and, secondly, that the kidneys are practically freed from the ever varying influence of the tissues, and that the composition of the blood can be kept constant, or can be altered as desired. The preparation offers, therefore, a means of investigating independently the part taken on the one hand by circulatory changes, and on the other hand by the composition of the blood bringing about the flow of urine.

SUMMARY.

A method for perfusing the isolated kidney with arterial blood from the heart is described. Under these conditions the arterial blood-pressure, blood flow and temperature are under control, and the gaseous exchanges of the kidney can be investigated.

The kidney thus isolated is capable of secreting urine.

The gaseous metabolism of the isolated kidney is increased when the urine secretion is provoked by the aid of diuretics such as sodium sulphate. The respiratory quotient of the kidney is similar to that of the heart.

The gaseous metabolism of the kidney is not altered by changes in the rate of blood flow through the organ.

The expenses of this research have been defrayed by a grant from the Royal Society.

REFERENCES.

(1) **Barcroft and Brodie.** This Journal, XXXIII. p. 52. 1905.
(2) **Barcroft and Müller.** Ibid. XLIV. p. 259. 1912.
(3) **Barcroft and Straub.** Ibid. XLI. p. 145. 1910.
(4) **Evans.** Ibid. XLV. p. 213. 1912.
(5) **Knowlton and Starling.** Ibid. XLIV. p. 206. 1912.
(6) **Evans.** Ibid. XLVII. p. 409. 1914.

THE FERMENTS OF THE PANCREAS. PART IV. STEAPSIN. By JOHN MELLANBY, M.D., AND V. J. WOOLLEY, M.D.

(*From the Physiological Laboratory, St Thomas's Hospital.*)

CONTENTS.

	PAGE
The stability of steapsin	288
Destruction of steapsin by trypsin	290
Influence of electrolytes and bile salts on the digestion of fat by steapsin	293
The question of a coenzyme to steapsin	294
Discussion of results	298

Introduction.

IN 1891 Rachford(1) investigated the properties of steapsin contained in pancreatic juice obtained from a rabbit by means of a fistula. He considered himself fortunate if he obtained 1 c.c. of juice from any one rabbit. But despite this fact he was able to show that bile increased the action of steapsin threefold, that the velocity of the reaction was doubled by raising the temperature from 18° C. to 37° C., and that the action of the ferment was retarded by hydrochloric acid. In 1910 an extensive series of researches on the steapsin of pancreatic juice, obtained by means of secretin, were published by Terroine(2). He found that the ferment was destroyed after heating the juice to 60° C. for ten minutes and that its activity was greatly diminished after heating to 50° C. for forty-five minutes; that pancreatic juice added to olive oil immediately after activation hydrolysed the fat to a considerable extent; that the same juice five hours after activation contained practically no steapsin; and that the addition of coagulated egg white to active juice preserved the steapsin to a considerable degree. The coenzyme of steapsin has been investigated by Rosenheim and Shaw Mackenzie(3). They have found that serum has a well-marked accelerating action on lipase contained in a glycerine extract of the pancreas, a fact which has been confirmed by Miniami(4). Hamsik(5) has shown that in order to obtain an active lipase from fresh pancreas the glycerine extract must be turbid, but that if dried pancreatic tissue be used a clear glycerine extract possesses well-marked fat splitting activity.

The quantitative estimation of steapsin. The amount of ferment present in a solution was estimated by determining the rate at which emulsified olive oil was hydrolysed by it. An emulsion containing 50 °/₀ olive oil was made by means of tragacanth mucilage. To 2 c.c. of this 50 °/₀ olive oil emulsion the ferment solution and water were added to bring the volume to 3 c.c. The mixture was incubated at 40° C. for one hour. After this time 3 c.c. of alcohol and two drops of a 1 °/₀ solution of phenol phthalein were added to it. The addition of alcohol to this extent has been shown by Kanitz(6) to facilitate the titration of the fatty acids. The degree of acidity generated by the steapsin was determined by titration against NaOH (·1 N), a correction being made, by means of a control experiment, for the original acidity of the oil emulsion. The rate of hydrolysis of this oil emulsion by the steapsin contained in varying quantities of fresh pancreatic juice is shown in the following figures.

Olive oil (50 °/₀)	H_2O	P. J.	NaOH (·1 N) required for neutralisation after one hour
2	·9	·1	1·4 c.c.
2	·8	·2	2·5
2	·7	·3	2·8
2	·6	·4	3·5
2	·5	·5	3·8

The above figures show that the amount of fat hydrolysed in an hour is not directly proportional to the amount of ferment added. From the curve expressing the relation between the amount of juice added and the amount of fatty acid formed the relative amounts of ferment in any of the following experiments may be calculated. In the majority of cases however the amount of steapsin present in a solution has been expressed in terms of the number of c.c. of NaOH (·1 N) required to neutralise the fat digestion mixture after one hour's incubation at 40° C.

The stability of steapsin.

The rate of destruction of steapsin varies with the conditions under which it may be placed. In order to analyse the effects observed a number of experiments were made on fresh pancreatic juice under varying conditions of reaction and temperature.

Alkali. Pancreatic juice of an alkalinity approximately Na_2CO_3 (·12 N) was left at 40° C. for five hours. At the end of each hour the lipolytic power of the juice was determined. The following numbers were obtained.

	At once	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
Steapsin in terms of NaOH (·1 N)	3·8	3·5	2·9	2·6	2·3	1·8 c.c.

The figures show a progressive destruction of steapsin—after heating the juice to 40° C. for five hours two-thirds of the ferment had disappeared. During this time no trypsin was produced in the juice. The trypsinogen was not diminished in any degree since on adding a small quantity of enterokinase to the portion of juice remaining after the five hours' heating a tryptic activity of one thousand units was developed. The significance of this fact will be evident later when we discuss the relation of trypsin to steapsin. At present it may be noted that pancreatic juice heated to 40° C. loses steapsin at the rate of 10 % per hour whilst trypsinogen is unaffected by this treatment.

Neutral solution. The destruction of steapsin in neutral solution at 40° C. occurs at practically the same rate as in alkaline solution. Pancreatic juice was neutralised by the addition to it of an equal quantity of HCl (·1 N). The following figures indicate the amounts of steapsin contained in ·1 c.c. of the neutralised and diluted juice after varying intervals of time.

	At once	1 hr.	2 hrs.	3 hrs.
Steapsin in terms of NaOH (·1 N)	2·5	2·2	2·0	1·7 c.c.

The rate of destruction is only a little slower than in pancreatic juice which has not been neutralised.

Acid. Steapsin is fairly stable at room temperature (16° C.) in alkaline or neutral solution but the presence of a minute quantity of free mineral acid rapidly destroys it. The following experiment illustrates this statement.

(A) ·5 c.c. P. J. + ·8 c.c. HCl (·1 N) (slightly acidic).
(B) 5 c.c. P. J. + ·8 c.c. H_2O.

After fifteen minutes at 40° C. 2 c.c. of a 50 % olive oil emulsion were added to each of these solutions. After one hour's incubation at 40° C. the acid developed in each tube required for neutralisation (A) 1 c.c. NaOH (·1 N) and (B) 5 c.c. NaOH (·1 N).

The presence of a small quantity of free HCl in the juice (approximately HCl ·02 N) diminished the lipolytic activity to less than one-fifth of its former value. Experiments, detailed later, show that electrolytes do not affect the action of steapsin on fat. We must therefore conclude that HCl ·02 N actively destroys this ferment.

The presence of organic acids to the extent of about ·2 N in the above fat digestion mixture (B) indicates the stability of steapsin in the presence of organic acids. Apparently the stability of the ferment in acidic solution is related to the hydrogen ion concentration of the solution. We propose to deal with this problem in a subsequent paper.

Temperature. It has been shown that steapsin is gradually destroyed when pancreatic juice is heated to 40° C., the rate of destruction being approximately 10 % per hour. The following experimental results show that this rate of destruction is considerably augmented at 50° C.

Period of heating to 50° C.	Activity of juice in terms of NaOH (·1 N)
0 mins.	3·5 c.c.
15	1·7
30	1·0
45	·5
60	·1
75	0

From these figures it may be seen that within fifteen minutes more than 50 % of the steapsin disappeared from pancreatic juice heated to 50° C. This rapid destruction of steapsin in pancreatic juice heated to 50° C., confirms the results obtained by Terroine (2) and is comparable to that described by us in the case of trypsin. In the case of this latter ferment the whole of it contained in activated pancreatic juice is destroyed by heating to 50° C. for forty-five minutes. Further experiments showed that steapsin is completely destroyed by heating pancreatic juice to 60° C. for five minutes. A similar temperature of destruction has been shown to hold good for trypsin also.

The relation of steapsin to trypsin.

From the experiments of Terroine (2) it appeared that steapsin is unstable in the presence of trypsin. Our method of estimating tryptic activity has enabled us to show in detail that as the tryptic power of pancreatic juice develops the lipolytic activity diminishes. This disappearance of steapsin with the development of trypsin is illustrated in the following experimental results.

Pancreatic juice was neutralised by the addition to it of $CaCl_2$ (·1 N), and allowed to become active. After varying times the lipolytic and tryptic activities of the juice were determined.

	Activity after					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
Steapsin in terms of NaOH (·1 N)	4·0	3·7	3·5	·2	0	0
Units of trypsin	0	0	0	660	1250	1000

From these figures it may be seen that in the first three hours no trypsin was developed and the steapsin remained practically intact. In the fourth hour 600 units of trypsin were produced and the steapsin disappeared.

Many experiments have conclusively shown that in fresh pancreatic juice steapsin is present in large quantities. On the addition of enterokinase to the juice steapsin persists only so long as minute quantities of trypsin are present. When full proteolytic activity is developed the steapsin entirely disappears.

The rapid destruction of steapsin by trypsin is illustrated in the following experiment.

To 1 c.c. of fresh pancreatic juice ·2 c.c. of enterokinase was added and the lipolytic and tryptic activities determined at once and after twenty minutes' incubation at 40° C.

	At once	After 20 mins.
Steapsin in terms of NaOH (·1 N)	3·8	·2 c.c.
Units of trypsin	0	1000

Within twenty minutes the juice lost the whole of its steapsin and developed full proteolytic activity.

The following experiment differentiates between the gradual destruction of steapsin in pancreatic juice heated to 40° C. and that caused by trypsin. For a period of five hours juice was heated to 40° C. and its fat splitting and proteolytic activities determined every hour. At the end of this time enterokinase was added to the remaining juice and the activities of the mixture determined after a lapse of an additional thirty minutes.

		Activity after					
	At once	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	5½ hrs.
Steapsin in terms of NaOH (·1 N)	3·8	3·5	2·9	2·6	2·3	1·8	·2 c.c.
Units of trypsin	0	0	0	0	0	0	1000

Up to five hours the steapsin disappeared at the rate of 10 °/₀ per hour; after the addition of enterokinase full proteolytic activity was developed and the remaining steapsin was destroyed within thirty minutes.

These experimental results show conclusively that steapsin cannot exist in the presence of trypsin. They afford an adequate explanation of the observation that pancreatic juice which contains trypsin is devoid of fat splitting activity. The importance of the fact in relation to fat digestion will be discussed in the concluding section of this paper.

The influence of serum and egg albumen. The following mixtures of pancreatic juice, ox serum, and enterokinase were made up:

P. J.	E.	Ox serum	H_2O	
·5	·0	·5	·0 c.c.	(A)
·5	·05	·45	·0	(B)
·5	·05	·0	·45	(C)

After an hour's incubation at 40° C. (C) contained a large quantity of trypsin but no steapsin, whilst (A) and (B) contained no trypsin but an undiminished quantity of steapsin. The hypothesis which we put forward to account for the preservation of steapsin is that the antitrypsin present in the serum neutralised the trypsin as it was produced and so prevented the destruction of steapsin. This hypothesis was confirmed by determining the preservative action of egg albumen on steapsin.

The following mixtures of fresh pancreatic juice, enterokinase and albumen were made up:

P. J.	E_{10}	H_2O	Alb.	
·5	·1	·35	·05 c.c.	(A)
·5	·1	·4	0	(B)

After two hours (A) was devoid of proteolytic activity whilst (B) contained a large quantity of trypsin. At the same time the lipolytic activities of (A) and (B) were compared with that of a similar quantity of fresh pancreatic juice.

	Oil emulsion (50 %)	H_2O	P. J.		NaOH (·1 N) required after one hour
(Control)	2	·9	·1		3·0 c.c.
	2	·8	·2	(A)	2·6
	2	·8	·2	(B)	·1

Comparing the fat splitting powers of (A) and (B) with that of the control it may be seen that the presence of egg albumen (A) had preserved the steapsin almost intact, whilst in experiment (B), in which enterokinase only had been added to the juice, the steapsin had disappeared. In the one tube, (A), the added egg albumen neutralised the trypsin and thereby preserved the steapsin. In the other tube, (B), to which no albumen had been added, a large quantity of trypsin was produced and the steapsin was destroyed.

We may conclude therefore that the preservative actions exerted by serum and egg albumen on the steapsin of pancreatic juice are due to the antitrypsins contained in them. Steapsin cannot exist in the presence of free trypsin. If the development of trypsin in pancreatic juice be prevented, or if the trypsin when first formed be neutralised by antitrypsin, then the steapsin remains intact. This observation, also, is of fundamental importance in considering the digestion of fat in the small intestine.

The influence of electrolytes and bile salts on the digestion of fat by steapsin.

Briefly stated, bile salts augment to a marked degree the digestion of fat by steapsin, but electrolytes have practically no effect on this hydrolysis. These general statements are illustrated in the following experiments:

Sodium glycocholate in varying amounts, and ·1 c.c. of fresh pancreatic juice, were added to 2 c.c. of a 50 % olive oil emulsion and the degree of acidity developed after an hour's incubation at 40° C. was determined by titration against NaOH (·1 N).

Olive oil emulsion (50 %)	H_2O	P. J.	Sodium glycocholate (5 %)	NaOH (·1 N) required after one hour
(A) 2	·9	·1	·0	3·6 c.c.
(B) 2	·0	·1	·9	6·8

The presence of 1·5 % sodium glycocholate more than doubled the amount of oil hydrolysed in one hour by the steapsin contained in ·1 c.c. of pancreatic juice. Exp. (B) illustrates the remarkable lipolytic activity of fresh pancreatic juice in the presence of bile salts. A mixture containing only 3 % of pancreatic juice hydrolysed a 33 % olive oil emulsion at such a rate that organic acid to the extent of ·2 N was developed in an hour.

Similar results were produced by the addition of bile to the digestion mixture.

Olive oil	P. J.	H_2O	Bile	NaOH (·1 N) required after one hour
2	·1	·7	·2	5·0 c.c.
2	·1	·9	·0	3·8
2	·0	·8	·2	·0

From these figures it may be seen that whereas bile itself had no action on the oil yet a similar quantity of bile (6 % approximately) acting in conjunction with steapsin doubled the amount of oil hydrolysed in one hour.

The marked action of bile salts on steapsin is in striking contrast with the absence of effect of electrolytes on this ferment. The following figures show the rate of digestion of olive oil by fresh pancreatic juice, and by similar quantities of juice in the presence of various electrolytes.

Olive oil emulsion (50 %)	H_2O	P. J.	Electrolyte	NaOH (·1 N) after 1 hr.
2	·9	·1	0	3·5 c.c.
2	·8	·1	·1 HCl (·01 N)	3·3
2	·8	·1	·1 NaOH (·01 N)	3·3
2	·8	·1	·1 NaCl (N)	3·8
2	·8	·1	·1 $CaCl_2$ (N)	3·3
2	·8	·1	·1 $MgCl_2$ (N)	3·1

These results are opposed to those of **Terroine**(2) who finds that electrolytes augment the action of steapsin on fat. The small effects which we have noted may appear to support **Terroine's** work but we think that they must be ascribed to experimental error and possibly the effect of the electrolyte on the permanence and degree of the oil emulsion rather than to any adjuvant action on the lipase. Possibly the effect of the electrolytes on the permanence of the fat emulsion is important since **Terroine** working with unemulsified olive oil found that cane sugar augmented fat digestion tenfold. We have been unable to confirm this remarkable result working with emulsified oil. The addition of cane sugar to a mixture of 50 % olive oil emulsion and pancreatic juice neither augments nor diminishes the degree of fat digestion. Since the only factor in which our experiments differed from those of **Terroine** consisted in the emulsification of the oil it is clear that the extraordinary adjuvant action exerted by cane sugar in the digestion of olive oil by steapsin must be ascribed to the emulsifying property of the added syrup. But apart from the question of the effect of cane sugar on fat digestion it is evident that the influence exerted by electrolytes on steapsin is by no means comparable to the effects produced by these substances on amylopsin. In this latter case the achromic time for a mixture of pancreatic juice and starch may be extended tenfold or diminished to a corresponding degree by the addition of certain electrolytes in suitable concentrations.

The question of a coenzyme to steapsin.

Ten years ago **Magnus**(7) described experiments by which he separated the lipase of liver into two components, neither of which alone was capable of hydrolysing fat, but which, when added to one another, produced the activity of the original extract. The one component possessed the properties of the original ferment solution as regards heat destruction, etc., the other component was dialysable, heat stable at 100° C., and soluble in alcohol. To this latter component the name coenzyme was given. **Loewenhart**(8) found that bile salts possessed all the properties of this coenzyme of liver lipase. **Rosenheim** and **Shaw Mackenzie**(3) found that steapsin contained in a glycerine extract of the pancreas could be separated into two inactive components by precipitation with a large volume of water, the one component being found in the precipitate, the other component (the coenzyme) being present in the filtrate. Further **Rosenheim** and **Shaw Mackenzie** found that serum had a well-marked accelerating action on pancreatic lipase similar to that shown by the above-mentioned coenzyme.

We may say at the outset that we have been unable to confirm these results as regards the steapsin contained in fresh pancreatic juice. Owing to the fact that dialysis occupies a considerable time, and that dialysed pancreatic juice develops a proteolytic activity with consequent disappearance of the steapsin, we have been unable to adopt the method originally employed by Magnus with liver lipase. We have, however, employed a method which is completely successful in separating amylopsin of pancreatic juice into enzyme and coenzyme, namely, by precipitating fresh pancreatic juice with 95 °/o of absolute alcohol. By this method we find that the enzyme component of amylopsin is contained in the precipitate and the coenzyme in the filtrate. If pancreatic lipase be similar to liver lipase this method ought to separate it into two components since a striking property of the coenzyme of the liver lipase is its solubility in alcohol (cf. Magnus). The following gives the results of an experiment:

2 c.c. of fresh pancreatic juice were added to 30 c.c. of absolute alcohol cooled to −10° C. The resulting solution was centrifuged at a temperature below zero and the supernatant fluid was removed from the precipitate with a pipette. The precipitate, when dissolved in water, gave a clear solution. The activities of the original juice and the alcohol precipitate dissolved in a volume of water equal to that of the original juice were as follows:

Olive oil	H_2O	P. J.	NaOH (·1 N) required for neutralisation after one hour
2	·8	·2	3·5 c.c.
2	·8	·2 (X)	2·8

There was a small diminution in activity of the precipitated juice but this loss was due to the destruction of the steapsin by alcohol. The activity of the precipitate was not restored to its original value by adding the filtrate, freed from alcohol, to it. We have found no evidence that steapsin contained in pancreatic juice can be separated into enzyme and coenzyme from a number of similar experiments made with variations in the amounts of alcohol added, the time allowed for precipitation, and the temperature of the solutions.

We are the more inclined to assume that steapsin contained in pancreatic juice cannot be divided into two components corresponding to enzyme and coenzyme since we find that electrolytes neither augment nor diminish its activity to any degree. All the effects produced by the coenzyme of amylolytic ferments can be simulated by inorganic salts, such as sodium chloride or calcium chloride, and these salts have an extraordinary accelerating influence on the activities of ptyalin or amylopsin contained in solutions which have not been treated so as to separate them into their components. In fact it appears probable that fluids containing these enzymes as saliva and pancreatic juice do not contain an optimal quantity of coenzyme. By analogy it may be assumed that if steapsin has a similar constitution similar facts should hold good for it. In the absence of these facts we may deduce that the two types of ferments are not analogous and that steapsin contained in pancreatic juice is not formed of two components which can be separated from one another.

The question then arises as to the possible explanation of the differences between our experimental results obtained with steapsin contained in fresh pancreatic juice, and those described by other experimenters working with a glycerine extract of the pancreas. A most important point is the condition of the steapsin in the two preparations. In pancreatic juice the steapsin is held in complete solution and no amount of filtration diminishes the lipolytic activity of the juice. On the other hand the lipase contained in a glycerine extract of the pancreas is not held in solution since the fluid obtained after filtration through ordinary filter paper is practically devoid of any lipolytic action. In the preparation of such an extract of the pancreas it is essential that the resulting fluid should be turbid and not water clear, an end which is achieved by pressing the extracting mixture through muslin. We regard this suspensoid condition of the steapsin contained in glycerine extracts of the pancreas as responsible for the fact that the precipitate obtained from such an extract by dilution with water is devoid of lipolytic activity. The steapsin contained in the precipitate is insoluble in water (Hamsik) and it is essential that the ferment should at least approximate to a condition of solution before it can exert its specific action. This condition may be produced by the substances present in the filtrate after precipitation of the glycerine extract with water, or by various electrolytes such as alkaline carbonates, or by fluids containing these electrolytes in solution as serum.

The capacity of serum to simulate the action of a coenzyme is also evident from the experiments of Rosenheim and Shaw Mackenzie in which they showed that the addition of serum to a glycerine extract of the pancreas greatly augments the lipolytic activity. In this action two factors are probably involved: (*a*) the action discussed above, *i.e.* the more adequate solution of the lipase contained in the glycerine extract by certain components of the serum, and (*b*) the preservation of the steapsin from the action of trypsin by the antitrypsin contained in the added serum.

We have previously discussed the effect of serum on the destruction of steapsin by trypsin, the only question which now arises being whether a glycerine extract of the pancreas contains trypsin or enterokinase and trypsinogen, in addition to steapsin.

At the outset it may be noted that the addition of serum to fresh inactive pancreatic juice has no effect whatever upon its lipolytic activity. Fresh pancreatic juice diluted with water hydrolyses as much olive oil emulsion in an hour as a similar quantity of fresh pancreatic juice diluted with serum. Such an experiment effectively dismisses the possibility of the existence of a coenzyme in serum for steapsin contained in fresh pancreatic juice. (It is essential that this juice should not contain more than a trace of enterokinase.) A glycerine extract of the pancreas however contains not only steapsin but also trypsinogen and enterokinase. We (11) have previously shown that the slow development of trypsin and the continued existence of steapsin in such a solution is due to the inhibitory action of strong glycerine on the activation of trypsinogen by enterokinase, and on the proteolytic activity of trypsin. Dilution of such an extract removes the inhibitory effects produced by the glycerine and there results activation of the trypsinogen by the enterokinase, and the subsequent disappearance of the steapsin at a rate depending upon the rate of trypsin production. The addition of the antitrypsin contained in serum to such a diluted glycerine extract neutralises the trypsin as it is produced and so permits the steapsin to act for the whole of the digestive period instead of for a time depending upon the rate of conversion of trypsinogen into trypsin. In this way serum simulates the action of a coenzyme merely by virtue of the antitrypsin contained in it.

Finally we have added serum and bile salts to pancreatic juice which has lost its lipolytic property owing to the development of trypsin within it. In no case has the added serum or bile salt generated steapsin, showing that the loss of lipolytic activity from pancreatic juice, produced by trypsin, is not due to the disappearance of a coenzyme but to an absolute destruction of the ferment itself.

Discussion of results.

From the experimental results described in the previous pages two main questions arise for discussion: (*a*) the properties of steapsin, and (*b*) the mechanism of fat digestion in the small intestine.

(*a*) *The properties of steapsin.* The problem as to the ultimate nature of ferments has given rise to much speculation and experimental work. The greater part of the work has been directed towards the purification of ferment solutions and analysing the final products. The majority of workers have concluded that ferments consist essentially of protein. The main criticism against this conclusion is that the protein tests are given by substances associated with the ferment, rather than by the ferment itself.

The relation of trypsin to steapsin appears to settle this problem conclusively so far as steapsin is concerned. It has been fully proved that so soon as trypsin develops in pancreatic juice steapsin disappears. The fact admits of only one explanation—that steapsin is digested by trypsin, and since trypsin is a proteolytic ferment then steapsin consists essentially of protein.

A second fact observed in the properties of steapsin is that the stability of this ferment in alkaline or neutral solution at any temperature is approximately the same as trypsin (always assuming that no trypsin is present in the solution). The rapid disappearance of trypsin from solution has been assumed to be due to autodigestion. We have previously put forward arguments against this assumption and have indicated that its disappearance is due to inherent instability. These arguments receive considerable support from the observations stated above: that the stability of steapsin in alkaline or neutral solution is approximately the same as trypsin. It is in the highest degree improbable that steapsin which consists essentially of protein should undergo autodigestion since its function is to hydrolyse fat. And arguing from these facts we may legitimately conclude that the disappearance of trypsin from solution is not due to autodigestion but to its inherent instability.

Another point which arises from the relation of steapsin to trypsin is the question of the identity of the milk clotting ferment of pancreatic juice with trypsin. This problem has been discussed by one (9) of us in considerable detail, and we concluded from a critical survey of the subject that pancreatic rennin and pancreatic trypsin are identical with one another—in fact that the coagulation of milk by trypsin is an expression of a general law that all proteolytic ferments coagulate milk provided an adequate amount of calcium be contained in it. Exception has been taken to this hypothesis by Edie(10) who finds that trypsin, as estimated by its capacity to hydrolyse protein, is preserved to a somewhat greater extent after boiling in acidic solution than is denoted by the figures which we have given in this connection. The unique fact that the ferment or ferments in pancreatic juice which digest protein and coagulate milk should withstand boiling in acidic solution is practically conclusive proof that the two actions are produced by one and the same substance. But as additional evidence in favour of the identity of the milk clotting ferment with trypsin we offer this relation of the milk clotting ferment to steapsin. If pancreatic rennin exists as a separate ferment then the disappearance of steapsin from pancreatic juice when rennin develops is unintelligible. If however pancreatic rennin and trypsin are identical then the disappearance of steapsin from activated pancreatic juice receives a ready explanation.

(b) *The mechanism of fat digestion in the small intestine.* The relation of steapsin to trypsin throws some light on the mechanism of fat digestion in the small intestine. Since steapsin is quickly destroyed by trypsin it is essential that the digestion of fat should take place either in the absence of trypsin, or, if present, under such conditions that its destructive action on steapsin is prevented. The rate of activation of trypsinogen by enterokinase is admirably adapted to secure an adequate fat digestion in the upper part of the small intestine. We(11) have previously investigated this phenomenon in detail: suffice it to say that at the beginning of the reaction the rate of activation of trypsinogen is very slow, but as the change proceeds this rate is continually accelerated until finally the velocity is relatively large. From this it follows that steapsin may remain intact and fat digestion may take place in the upper part of the small intestine during the time occupied by the initial stages of trypsinogen activation.

The question next arises as to the conditions which augment or diminish the rate of trypsinogen activation. We have previously shown that the rate of trypsinogen activation is directly proportional to the amount of enterokinase present, and that the reaction proceeds most rapidly in neutral solution, is inhibited by alkalies, and stopped by acids. A consideration of these facts enables a deduction to be made as to the optimal conditions for fat digestion in the small intestine.

Enterokinase is contained in the succus entericus, and the upper two-fifths of the small intestine contain much more enterokinase than the remaining portion of the gut. The active factor involved in the secretion of succus entericus is stated to be the mechanical excitation of the intestine by the food contained in it. Consequently the secretion of enterokinase will be small and fat digestion facilitated on a diet which contains a minimum of material capable of mechanically exciting the mucous membrane of the upper part of the small intestine.

The reaction of the contents of the small intestine appears to play a less important part in the digestion of fat than appeared probable from the early determinations of the alkalinity of pancreatic juice. According to many observers the alkalinity of pancreatic juice varies between Na_2CO_3 (·1 N) and Na_2CO_3 (·2 N). Auerbach and Pick (12), however, have recently shown that pancreatic juice, secreted from a fistula, is practically neutral in reaction, since the juice is saturated with CO_2, the alkali thus existing as $NaHCO_3$ and not as Na_2CO_3. The well-marked alkalinity ascribed by earlier workers may be explained on the assumption that their determinations were made on old samples of juice which had lost their CO_2. Our experiments fully confirm those of Auerbach and Pick. Fresh pancreatic juice obtained by means of secretin has a hydrogen ion concentration which corresponds to NaHCO (·2 N) and which is only slightly alkaline to phenōl-phthalein. Succus entericus also is only slightly alkaline in reaction. It may be assumed therefore that the contents of the small intestine are approximately neutral in reaction, and that the conditions as regards reaction are such as to determine a maximum rate of trypsinogen activation by enterokinase.

The destruction of enterokinase by hydrochloric acid may be dismissed as outside the range of physiological action since the acid control of the pylorus prevents the contents of the small intestine from becoming acidic. In any case, free acid, to the extent of HCl (·02N) in the small intestine, would not only prevent the activation of trypsinogen but would also destroy any steapsin and amylopsin with which it came in contact.

Finally, a factor which may exert a marked effect on fat digestion is the amount of protein contained in the diet. The protective action of egg albumen on the steapsin of pancreatic juice has been considered in detail. This protective action depends mainly upon the adsorption of the trypsin by the egg albumen and the degree of protection depends upon the rate at which the albumen is digested by the attached trypsin. From this it follows that fat in a dietary containing protein is more favourably placed for digestion by steapsin than fat taken alone or in conjunction with carbohydrate.

However, apart from the factors which determine the optimal conditions for the digestion of fat, one thing is clear—in the upper part of the small intestine fat is digested by steapsin and trypsinogen is converted into trypsin by enterokinase. When free trypsin exists in the intestinal contents, the steapsin disappears and fat digestion ceases.

Finally the relation of trypsin to steapsin offers an adequate explanation for the presence of trypsinogen rather than trypsin in fresh pancreatic juice.

SUMMARY.

(1) The stability of steapsin in alkaline solution is similar to that of trypsin. Fresh pancreatic juice at 40° C. loses steapsin at the rate of 10 % per hour; at 50° C. this rate is augmented to 50 % per hour; and at 60° C. the whole of it is destroyed within five minutes.

(2) The stability of steapsin in acidic solution is related to the hydrogen ion concentration of the fluid in which it is contained. It is stable in the presence of large quantities of the higher fatty acids (·2 N) but minute quantities of free mineral acids (HCl ·02 N) quickly destroy it.

(3) Steapsin cannot exist in the presence of free trypsin. Therefore, when pancreatic juice is activated by enterokinase, as trypsin develops steapsin disappears. This fact affords an explanation for the presence of trypsinogen rather than trypsin in fresh pancreatic juice.

(4) Serum, or egg albumen, added to activating pancreatic juice, protects steapsin from destruction owing to the presence of antitrypsin in them.

(5) The action of steapsin on fat is greatly augmented by bile and bile salts. Electrolytes, such as neutral salts, have no influence on the reaction.

(6) There is no evidence that steapsin in pancreatic juice can be separated into enzyme and coenzyme. Serum, when added to pancreatic extracts, may simulate the action of a coenzyme owing to the antitrypsin contained in it protecting the steapsin from any tryptic action.

(7) Steapsin cannot be restored to activated pancreatic juice by the addition of serum or bile. The destruction of steapsin by trypsin is absolute.

(8) From a consideration of the properties of steapsin and its relation to trypsin it appears:

(*a*) that steapsin consists essentially of protein; (*b*) that the destruction of trypsin in alkaline solution is not due to autodigestion but to its inherent instability; (*c*) that although the conditions in the small intestine which favour trypsin production are inimical to the continued existence of steapsin, yet the presence of protein in a dietary may facilitate fat digestion by virtue of the capacity of the protein to adsorb the first formed trypsin.

The expenses of this research have been defrayed by a grant from the Royal Society.

REFERENCES.

(1) **Rachford.** This Journal, xii. p. 72. 1891.
(2) **Terroine.** Biochem. Ztsch. xxiii. p. 404. 1910.
(3) **Rosenheim and Shaw Mackenzie.** This Journal, xl. Proc. viii–xiii. 1910.
(4) **Miniami.** Biochem. Ztsch. xxxix. p. 392. 1912.
(5) **Hamsik.** Ztsch. f. physiol. Chem. lxxi. p. 238. 1911.
(6) **Kanitz.** Ber. d. Deutsch. chem. Ges. 1903, p. 400.
(7) **Magnus.** Ztsch. f. physiol. Chem. xli. p. 149. 1904.
(8) **Lowenhart.** Amer. Journ. Physiol. xv. Proc. xxvii. 1905.
(9) **Mellanby.** This Journal, xlv. p. 345. 1912.
(10) **Edie.** Biochem. Journ. viii. p. 84. 1914.
(11) **Mellanby and Woolley.** This Journal, xlvii. p. 339. 1913.
(12) **Auerbach and Pick.** Arbeiten a. d. k. Gesundheitsamte, xliii. p. 155. 1912.

THE SENSITIVENESS OF THE RESPIRATORY CENTRE TO CARBONIC ACID, AND THE DEAD SPACE DURING HYPERPNŒA. BY J. M. H. CAMPBELL, C. GORDON DOUGLAS AND F. G. HOBSON.

(*From the Physiological Laboratory, Oxford.*)

IN a recent paper[1] attention has been drawn to the great sensitiveness of the respiratory centre to CO_2. The actual experiments described in that paper indicated that a rise of about 0·3 % in the alveolar CO_2-pressure was sufficient to cause the alveolar ventilation of the lungs to be increased by 100 % above the resting value. At the same time arguments were advanced to show that in all probability this observed value was really too high, as, owing to the technique adopted, it was impossible to make allowance for any increase in the metabolism during the experiments, the alteration in the alveolar ventilation being deduced from a comparison of the differences of the concentration of CO_2 in the alveolar and inspired airs.

Supposing that the metabolism had been increased while air containing CO_2 was being breathed the actual hyperpnœa would have been greater than the calculated value indicated, and it was finally concluded that a rise of about 0·22 % or 1·6 mm. of CO_2-pressure in the alveolar air was really sufficient to cause an increase of 100 % in the alveolar ventilation. It was also shown that erroneous conclusions were likely to be drawn if the observations were made before the body had got into equilibrium with the particular CO_2 mixture breathed.

In the course of some other experiments it became necessary to verify these conclusions as to the sensitiveness of the respiratory centre to CO_2, and to obtain some more precise quantitative relationship between the alveolar ventilation and the alveolar CO_2-pressure than is implied by the somewhat casual expression of 100 % increase above the resting ventilation.

---

[1] Campbell, Douglas, Haldane and Hobson. This *Journal*, XLVII. p. 301. 1913.

Our experiments were made in an air-tight chamber of about 250 cub. ft. or 7000 litres capacity. The size of this chamber was sufficient to enable the observer to manipulate all the necessary apparatus while the subject of the experiment, who was in all cases C. G. D., remained sitting at rest in a deck chair. Two series of experiments were made in atmospheres containing different amounts of CO_2 so as to cause different degrees of hyperpnœa.

In the first series of experiments (nos. 1 to 3) our procedure was as follows. The subject first of all sat at rest in the chamber with the door open and an electric fan blowing fresh air into it. After the expiration of a quarter of an hour a pair of samples of the alveolar air was taken by the Haldane-Priestley method in vacuous tubes, and three minutes later a sample of the expired air was collected in a gas bag for the determination of the volume of air breathed and the total respiratory exchange by the Douglas method[1]. These two observations were repeated about 10 minutes later, *i.e.* about half an hour after entering the chamber. After this the door of the chamber was shut, and 11 cub. ft. of CO_2 were passed into it through a gas meter by the second observer who was stationed outside, thorough mixture of the CO_2 with the air in the chamber being ensured by the fan. The percentage of CO_2 in the air in the chamber was thereby raised to about 4·5 %, the oxygen percentage being reduced to about 19·8 %: during the course of the experiment the CO_2 percentage rose at the rate of about 0·6 % per hour, the oxygen percentage being correspondingly reduced. In order that the subject might get into full equilibrium with the mixture breathed a period of half an hour was allowed to elapse and then another pair of alveolar samples was taken, followed two minutes later by a sample to allow of the determination of the total respiratory exchange. About half an hour later, *i.e.* one hour after running the CO_2 into the chamber, these observations were repeated. A further period of about ten minutes was allowed to elapse and then another pair of alveolar samples was taken. After this the door of the chamber was opened and the fan so placed as to direct a large current of fresh air through the chamber. In two of the experiments two further pairs of alveolar air samples were taken 10 minutes and 20 minutes respectively after the door of the chamber was opened. The subject of the experiment remained seated throughout practically the whole of the experiment, and endeavoured to keep himself in a uniform condition of rest especially in the 15 minutes immediately preceding the collection of the samples. The samples of the expired air collected in the bag were measured in the chamber by the observer, all analyses being done subsequently after leaving the chamber. Samples of the inspired air were taken between the two sets of observations made before closing the chamber, immediately after taking each pair of alveolar samples (and therefore just before taking each sample for the determination of the total respiratory exchange) while the chamber was closed, and finally just after the first pair of alveolar samples had been taken after opening the chamber.

---

[1] This *Journal*, XLII. p. xvii. 1911.

In the second series of experiments (nos. 4–7) about 8½ cubic feet of CO_2 were passed into the chamber raising the initial CO_2 percentage to about 3½ °/₀, and there was therefore considerably less hyperpnœa than in the first series. We consequently modified the technique a little as the differences in the composition of the various air samples were likely to be smaller, and we were anxious to minimise the chances of error in taking the alveolar air samples.

Instead of taking a single alveolar sample in a vacuous tube we took four equal small samples from the last portion of four different deep expirations into the same gas sampling tube by allowing equal amounts of mercury, with which the tube had previously been filled, to run out. Separate composite samples were obtained from deep expirations given, as in the original Haldane-Priestley method, at the end of a normal inspiration and of a normal expiration. We felt that by this means we should obtain a fairer average composition of the alveolar air, and since there were only two analyses to be made instead of eight, as there would have been if we had followed the original Haldane-Priestley method, much labour was saved. In practice the deep expirations were given alternately at the end of a normal inspiration and of a normal expiration.

After taking a composite sample of the alveolar air and a sample for the determination of the total respiratory exchange before the chamber was closed we waited, as in the previous series, for 30 minutes before commencing observations in the atmosphere containing CO_2. We then took in succession a composite sample of the alveolar air, a sample for the determination of the total respiratory exchange, another composite sample of the alveolar air, and another sample for the respiratory exchange, allowing an interval of three minutes between the different samples. In experiments 6 and 7 further samples for determining the composition of the alveolar air and the respiratory exchange were taken commencing 20 minutes after opening the door of the chamber. As it took 12 minutes to collect the composite samples of the alveolar air, since time had to be allowed for the breathing to become normal after giving each deep expiration before giving the next one, we took samples of the inspired air in the middle of taking the samples of the alveolar air and of taking those for determining the respiratory exchange while the chamber was closed. The total time during which we breathed air containing CO_2 was about an hour and a quarter.

We purposely remained in the chamber for a considerable time after adding the CO_2 to the air in the hope of ensuring that we were in equilibrium with the mixture breathed before commencing the observations, for the first effect of breathing air rich in CO_2 is to cause a retention of CO_2 in the body, *i.e.* a diminished output of the gas from the body and consequently a smaller degree of hyperpnœa than would be obtained in the absence of such retention. As a consequence of this the respiratory quotient is at first very low and only regains its normal level slowly.

Table I contains the figures obtained for the total respiratory exchange while normal air was being breathed and during the hyperpnœa induced by the addition of CO_2 to the inspired air. In this and the succeeding tables the observations are arranged in the order in which they were made.

In Exps. 1 to 3 the respiratory quotients during the hyperpnœa are not materially different from those obtained while breathing normal air, with the exception of the last observation in Exp. 1 where there was a rise of the respiratory quotient. The subject had therefore attained equilibrium with the CO_2 mixture breathed. In Exps. 4 to 7, however, the respiratory quotients during the hyperpnœa were on the whole definitely lower than those obtained when breathing normal air, a fact which is suggestive of incomplete equilibrium, notwithstanding the long duration of the experiments. If this is true it might owe its explanation to the slight effect produced on the circulation rate by so small a rise in the alveolar CO_2-pressure and lung ventilation.

The total respiratory exchange was with few exceptions somewhat less during the hyperpnœa than during the preliminary periods. It is, however, not fair to compare from the point of view of the metabolism the values obtained during the early part of the experiments with those obtained during the time that the CO_2 mixture was being breathed, as the resting metabolism would tend to fall after sitting still for so long a time. This is confirmed by the low values obtained in Exps. 6 and 7 for the total respiratory exchange when normal air was again breathed at the close of the experiments.

TABLE I.

	Normal air			Air containing CO_2		
	Respiratory exchange in c.c. per min. at s.t.p.		Respiratory quotient	Respiratory exchange in c.c. per min. at s.t.p.		Respiratory quotient
Number	O_2	CO_2		O_2	CO_2	
1	314	260	0·828	—	—	—
	305	249	0·816	—	—	—
	—	—	—	352	291	0·826
	—	—	—	358	330	0·921
2	290	230	0·793	—	—	—
	276	212	0·768	—	—	—
	—	—	—	277	220	0·795
	—	—	—	279	219	0·785
3	329	261	0·794	—	—	—
	312	237	0·760	—	—	—
	—	—	—	304	234	0·770
	—	—	—	310	242	0·781
Average of 1–3	304	242	0·793	313	256	0·813
4	307	238	0·776	—	—	—
	—	—	—	345	235	0·681
	—	—	—	304	244	0·802
5	344	266	0·773	—	—	—
	—	—	—	304	217	0·714
	—	—	—	294	230	0·782
6	306	230	0·752	—	—	—
	—	—	—	296	217	0·733
	—	—	—	282	205	0·727
	280	225	0·804	—	—	—
7	303	251	0·828	—	—	—
	—	—	—	276	217	0·787
	—	—	—	277	208	0·751
	268	223	0·832	—	—	—
Average of 4–7	301	239	0·794	297	222	0·747

Table II shows the concentration of CO_2 in the inspired, expired and alveolar airs. The figures for the alveolar air represent the mean of the determinations made at the end of a normal inspiration and of a normal expiration. We have not thought it necessary to give these latter values separately, as the difference between them was but small, and of the same order of magnitude during the quiet breathing and the hyperpnœa. In Exps. 1 to 3 the samples taken at the end of expiration

TABLE II.

	Normal air				Air containing CO_2			
			Alveolar air				Alveolar air	
Number	Inspired CO_2%	Expired CO_2%	CO_2%	CO_2 pressure in mm. Hg	Inspired CO_2%	Expired CO_2%	CO_2%	CO_2 pressure in mm. Hg
1	0·11	3·49	5·73	40·8	—	—	—	—
	—	3·34	5·79	41·2	—	—	—	—
	—	—	—	—	4·74	5·66	6·26	44·6
	—	—	—	—	5·04	5·86	6·35	45·2
	—	—	—	—	5·17	—	6·34	45·1
2	0·06	3·19	5·49	39·0	—	—	—	—
	—	3·05	5·60	39·8	—	—	—	—
	—	—	—	—	4·86	5·67	6·16	43·8
	—	—	—	—	5·15	5·92	6·17	43·9
	—	—	—	—	5·28	—	6·23	44·3
	0·10	—	5·56	39·6	—	—	—	—
	—	—	5·61	39·9	—	—	—	—
3	0·04	3·47	5·57	40·1	—	—	—	—
	—	3·29	5·36	38·5	—	—	—	—
	—	—	—	—	4·71	5·63	5·98	43·0
	—	—	—	—	5·01	5·81	6·13	44·1
	—	—	—	—	5·17	—	6·02	43·3
	0·10	—	5·25	37·7	—	—	—	—
	—	—	5·38	38·7	—	—	—	—
Average of 1–3	0·09	3·31	5·53	39·5	5·01	5·76	6·18	44·1
4	0·05	3·20	5·77	40·8	—	—	—	—
	—	—	—	—	3·47	—	5·96	42·1
	—	—	—	—	3·59	5·03	—	—
	—	—	—	—	3·77	—	5·87	41·5
	—	—	—	—	3·77	5·19	—	—
5	0·06	3·51	5·77	40·1	—	—	—	—
	—	—	—	—	4·12	—	6·31	43·9
	—	—	—	—	4·28	5·71	—	—
	—	—	—	—	4·43	—	6·43	44·7
	—	—	—	—	4·51	5·86	—	—
6	0·07	3·40	5·73	39·8	—	—	—	—
	—	—	—	—	4·05	—	6·06	42·1
	—	—	—	—	4·18	5·58	—	—
	—	—	—	—	4·31	—	6·07	42·1
	—	—	—	—	4·45	5·70	—	—
	0·08	3·37	5·60	38·9	—	—	—	—
7	0·03	3·44	5·89	40·8	—	—	—	—
	—	—	—	—	3·75	—	6·03	41·8
	—	—	—	—	3·90	5·45	—	—
	—	—	—	—	4·03	—	6·10	42·3
	—	—	—	—	4·17	5·54	—	—
	0·08	3·29	5·67	39·3	—	—	—	—
Average of 4–7	0·06	3·37	5·74	40·0	4·05	5·51	6·10	42·6

contained on an average 0·13 % more CO_2 when breathing normal air and 0·10 % more CO_2 when breathing the CO_2 mixture than did the samples taken at the end of inspiration: in Exps. 4 to 7 these differences were respectively 0·15 % and 0·12 %. In Exps. 1 to 3 the average oxygen percentages were 20·88 %, 16·99 % and 14·35 % in the inspired, expired and alveolar air respectively, and 19·10 %, 18·24 % and 17·59 % whilst breathing the air containing CO_2: in Exps. 4 to 7 these values were 20·89 %, 16·90 % and 13·57 % whilst breathing normal air, and 19·35 %, 17·50 % and 16·58 % whilst breathing the air containing CO_2.

It will be seen that the effect of breathing air containing about 5 % of CO_2 was to cause a rise in the alveolar CO_2-pressure of about 4½ mm., and of breathing air containing about 4 % of CO_2 a rise of about 2½ mm. The oxygen percentage in the expired and alveolar airs was considerably raised above its normal value while breathing the air containing CO_2 owing to the hyperpnœa in spite of the fall in the inspired oxygen percentage[1].

In the first series of experiments the total ventilation of the lungs was quadrupled during the hyperpnœa, and in the second series slightly more than doubled. The figures for the total volume of air expired and for the rate of the breathing are given in Table III. All volumes are expressed at 37°, saturated with moisture and under the prevailing barometric pressure, *i.e.* under the conditions which hold good within the body. During the hyperpnœa the rate of the breathing was considerably accelerated in the first series, but only slightly altered in the second series.

So far as the supply of oxygen and the discharge of CO_2 are concerned the effectiveness of the respiration depends on the alveolar ventilation. The alveolar ventilation per minute is obtained by subtracting the volume of the dead space from the volume of an expiration, measured at 37° and saturated with moisture, and multiplying the result by the number of breaths per minute. The volume of the dead space is given by the volume of an expiration measured at 37° and saturated with moisture × (the CO_2 percentage in the alveolar air − the CO_2 percentage in the expired air) ÷ (the CO_2 percentage in the alveolar air − the CO_2 percentage in the inspired air).

Proceeding by this method **Douglas** and **Haldane**[2] determined the dead space during muscular exercise and found it to be considerably

---

[1] See **Loewy**, *Untersuch. u. d. Resp. u. Zirkul.*, Berlin, 1895.

[2] This *Journal*, XLV. p. 235. 1912.

TABLE III.

	Normal air			Air containing CO_2		
Number	Expired air per minute in litres	Breaths per minute	Volume of each breath in c.c.	Expired air per minute in litres	Breaths per minute	Volume of each breath in c.c.
1	9·32	17·7	526	—	—	—
	9·34	19·6	476	—	—	—
	—	—	—	38·8	30·4	1276
	—	—	—	48·9	32·4	1510
2	8·94	21·0	426	—	—	—
	8·60	21·2	406	—	—	—
	—	—	—	33·3	31·3	1064
	—	—	—	34·9	30·0	1163
3	9·12	18·3	498	—	—	—
	8·75	18·5	473	—	—	—
	—	—	—	30·9	26·5	1166
	—	—	—	37·0	27·5	1345
Average of 1–3	9·01	19·4	468	37·3	29·7	1254
4	9·20	18·9	487	—	—	—
	—	—	—	20·2	24·3	831
	—	—	—	21·1	21·9	964
5	9·60	16·9	568	—	—	—
	—	—	—	19·1	17·8	1071
	—	—	—	21·4	19·9	1075
6	8·61	16·2	532	—	—	—
	—	—	—	19·6	18·2	1076
	—	—	—	20·8	19·0	1095
	8·52	15·5	550	—	—	—
7	9·16	16·8	545	—	—	—
	—	—	—	17·6	17·9	983
	—	—	—	19·1	18·2	1050
	8·66	17·0	509	—	—	—
Average of 4–7	8·96	16·9	532	19·9	19·7	1018

increased. Krogh and Lindhard[1] have however recently criticised these experiments, and have themselves come to the conclusion as the result of employing another method that the dead space is unaltered during the hyperpnœa of muscular work.

Their main criticism is that during the sharp expiration which is necessary when sampling the alveolar air by the Haldane-Priestley method the CO_2 percentage in the alveolar air is rising. During muscular work, when the rate of CO_2 output from the body is greatly increased, the error thus introduced becomes of very appreciable importance, even though the sharp expiration does not occupy more than half a second. The consequence is that the mean alveolar CO_2 percentage during expiration is estimated too high by the Haldane-Priestley method during muscular work, and as this value forms the basis for the calculation of the volume of the dead space the error will entirely explain the apparent great increase of the dead space.

---

[1] This *Journal*, XLVII. p. 30. 1913. *Ibid.* p. 431. *Skand. Arch. f. Physiol.* XXX. p. 375. 1913.

We freely acknowledge the justice of Krogh and Lindhard's criticism that the Haldane-Priestley method fails to give correct values during muscular work, and it seems that the true mean alveolar CO_2-pressure under these circumstances must for the time remain a matter of speculation. We are, however, far from convinced that the method which they advocate for the determination of the dead space gives true results.

This method consists of taking a single measured breath of hydrogen and calculating the dead space from the volume of a subsequent expiration and the percentage of hydrogen in the inspired gas and in the expired and alveolar air. They have found that the distribution of the gas in the alveolar air after a single breath of hydrogen is not uniform, and their figures show that even at rest the calculated volume of the dead space shows very great variations according to the depth of the expiration and the composition of the gaseous mixture inspired. In order to obtain concordant results by their method it is necessary to limit the volume of the expiration. These facts seem to us likely to militate seriously against the chances of obtaining true values for the dead space, quite apart from the considerations mentioned below, and with limitation of the expiration any actual increase in the calibre of the respiratory passages may be masked owing to failure to wash out these passages completely with the small volume of air available.

After all, the effective dead space, as understood in physiology, is an abstraction, not an anatomical reality. The values of the dead space which we calculate are expressed as though we have to deal only with the air in the alveoli and with the air in the upper respiratory passages, both having certain definite and ascertainable compositions, and as though the air in the upper respiratory passages is moved *en bloc* in inspiration or expiration towards or away from the alveoli. Such an expression is convenient, but it conceals the factors which underlie our conception of the dead space—the calibre of the bronchial tubes, the different rates of flow of the axial and peripheral streams of air in these tubes, the character and degree of penetration of air into the alveoli or adjacent parts of the air passages, and the liberation of appreciable quantities of CO_2 in other parts of the air passages than the alveoli. When so many factors may be concerned it would seem that any method based on the introduction into the lungs of some gas which is foreign to the body must give dubious results.

Krogh and Lindhard however agree that during rest the Haldane-Priestley method gives the most accurate results for the mean percentage of CO_2 in the alveolar air, and we have therefore an opportunity of studying in our present series of experiments the behaviour of the dead space during hyperpnœa when the rate of the metabolism is practically identical during the quiet breathing and during the hyperpnœa.

Table IV shows the values we have obtained for the dead space and the alveolar ventilation.

In Exps. 1 to 3 these values are calculated from the individual determinations of the volume of air breathed and the corresponding inspired, expired and alveolar analyses in those cases where all the data are complete. In Exp. 2 the alveolar CO_2-pressure was practically the same both before and after exposure to the atmosphere containing CO_2, but in Exp. 3 the alveolar CO_2-pressure whilst breathing normal air was slightly lower at the close of the experiment than it was at the commencement. If the dead space is calculated in the latter experiment from the mean volume of each breath at the commencement of the experiment and from all four alveolar CO_2 determinations made whilst breathing normal air it gives the value of 132 c.c.

In Exps. 4 to 7 it is rather more difficult to obtain the true value for the dead space during the hyperpnœa, since, owing to the time taken to collect the composite samples of alveolar air, a sufficient interval of time separated the collection of these samples from that of the samples for determining the respiratory exchange to cause a material alteration in the composition of the inspired air. The fairest way to proceed seems to be (1) to take the mean of the two alveolar CO_2 percentages and to calculate the dead space from the figure so obtained and the results of the first determination of the total respiratory exchange: (2) to calculate the dead space from the mean results of the two determinations of the respiratory exchange and the second determination of the alveolar CO_2 percentage. In this way we obtain in each case values for the inspired air for comparison with the alveolar air or total respiratory exchange, which are either identical or practically identical. The only exception to this close agreement is found in the second calculation in Exp. 4, where however the occurrence of the two

TABLE IV.

	Normal air		Air containing CO_2			
					Rise of alveolar CO_2-pressure in mm. Hg required to give a rise of 10 litres in	
Number	Effective dead space in c.c.	Alveolar ventilation in litres per min.	Effective dead space in c.c.	Alveolar ventilation in litres per min.	Alveolar ventilation	Total ventilation
1	158	5·59	—	—	—	—
	154	5·29	—	—	—	—
	—	—	452	25·1	2·1	1·2
	—	—	513	30·6	1·7	1·1
2	128	5·16	—	—	—	—
	135	4·65	—	—	—	—
	—	—	349	20·8	2·8	1·8
	—	—	233	26·4	2·1	1·7
3	137	5·65	—	—	—	—
	132	5·35	—	—	—	—
	—	—	269	22·4	2·2	1·7
	—	—	332	26·4	2·3	1·7
Average of 1–3	141	5·28	358	25·3	2·2	1·6
4	167	5·07	—	—	—	—
	—	—	266 (259)	12·5	1·3	0·9
	—	—	259 (260)	13·6	0·8	0·6
5	173	5·80	—	—	—	—
	—	—	286 (241)	13·0	5·8	4·4
	—	—	286 (254)	13·9	5·7	4·3
6	167	5·07	—	—	—	—
	—	—	227 (205)	14·5	2·9	2·5
	—	—	215 (178)	15·2	2·7	2·3
	170	5·08	—	—	—	—
7	176	5·33	—	—	—	—
	—	—	229 (198)	12·6	2·7	2·3
	—	—	244 (232)	13·0	2·8	2·3
	165	4·96	—	—	—	—
Average of 4–7	170	5·22	252 (228)	13·5	3·1	2·5

similar values of 3·77 °/₀ of CO_2 in the third and fourth inspired air samples renders some error in the sampling practically certain. In practice we have taken as inspired air values those corresponding to the total respiratory exchange in this calculation. Thus in Exp. 5: the first figure for the dead space during the hyperpnœa is calculated from the volume of each breath, 1071 c.c., the CO_2 percentage in the inspired air, 4·28 °/₀, and in the expired air, 5·71 °/₀, as found during the first respiratory exchange experiment, and from the mean of the two alveolar CO_2 percentages, 6·37 °/₀: the effective personal dead space so calculated is 286 c.c. after allowing 52 c.c. for the dead space of the valves. The figures used for the second determination of the dead space are the second alveolar CO_2 percentage, 6·43 %, and the mean of the values found in the two respiratory exchange determinations, viz. volume of each breath, 1073 c.c., inspired CO_2 percentage, 4·40 %, expired CO_2 percentage, 5·79 %, giving an effective personal dead space of 286 c.c.

We have also tried calculating the dead space in the same experiments in another way, viz. from each alveolar CO_2 percentage and the corresponding inspired CO_2 percentage, and the total volume of each breath and the expired CO_2 percentage found in the succeeding respiratory exchange determination. This method of calculation must give lower results than the previous one since the alveolar and inspired CO_2 percentages will clearly be too low, or the expired CO_2 percentage too high for strict comparison. We give, however, the results of these calculations in brackets in Table IV, and it will be seen that they indicate the same alteration in sense, though the less in degree, during the hyperpnœa.

All the results agree in showing a very considerable increase in the dead space during the hyperpnœa, the average increase being 217 c.c. in Exps. 1 to 3 where the ventilation of the lungs was quadrupled, and 82 c.c. in Exps. 4 to 7 where the breathing was doubled. In spite of this increase in the dead space the relative increase during the hyperpnœa of the alveolar ventilation[1] is about 15 % greater than that of the total ventilation in each of the two series.

Though the alveolar ventilation is the factor which determines the supply of oxygen to the lungs and the removal of CO_2 from them the real index of the work entailed on the respiratory muscles, and therefore of the activity of the respiratory centre, is the total ventilation of the lungs which secures that alveolar ventilation. The total ventilation necessary to secure any given alveolar ventilation may, however, be a variable quantity, since it depends on the depth and frequency of the breathing and on the precise volume of the dead space.

In order therefore to express the sensitiveness of the respiratory centre we show in Table IV the rise of the alveolar CO_2-pressure which is required to cause a rise of 10 litres in both the alveolar and the total ventilation. In Exps. 1 to 3 these figures are obtained from a comparison of the mean values of the ventilation and alveolar CO_2-pressure observed in each experiment while breathing normal air with each of the values observed while breathing the CO_2 mixture in the corresponding experiment, only those observations being used where all the data are complete. In Exps. 4 to 7 the mean data obtained in normal air are compared with figures derived from the data during the hyperpnœa in the manner already described in discussing the dead space.

---

[1] In calculating the alveolar ventilation 52 c.c. must be added to the effective personal dead space shown in Table IV to allow for the dead space of the valves.

It will be seen that an increase of 10 litres in the alveolar ventilation was caused by a rise of 2·2 mm. in the alveolar CO_2-pressure in the first series, and of 3·1 mm. in the second series, while a rise of 10 litres in the total ventilation was caused by a rise of 1·6 mm. in the alveolar CO_2-pressure in the first series and of 2·5 mm. in the second series. The individual figures show a very fair agreement, considering the small differences worked with, with the exception of those in Exp. 5, where exceptionally high values were obtained. Unfortunately we did not in this experiment make any further observations after recommencing to breathe normal air. We were at first somewhat afraid that prolonged exposure to an atmosphere containing CO_2 might cause some alteration in the normal threshold stimulating value of the alveolar CO_2-pressure, and it was for this reason that we made further observations in some of the experiments after recommencing to breathe normal air. So far as these observations went any alteration produced in the normal alveolar CO_2-pressure was trifling. If we except Exp. 5 the average rise of alveolar CO_2-pressure required to cause a rise of 10 litres in either the alveolar or total ventilations is practically identical in both series of experiments, and we do not therefore feel justified in suggesting that there was any real difference in the sensitiveness of the respiratory centre in the two series.

Taking the average of all the experiments the rise of alveolar CO_2-pressure required to give a rise of 10 litres in the alveolar ventilation was 2·5 mm. (a figure which is in very close agreement with that which we obtained in the earlier experiments with **Haldane**), and in the total ventilation 2·0 mm., *i.e.* a rise in the CO_2 percentage in the dry alveolar air of 0·35 °/₀ and 0·28 °/₀. Judging from **Hasselbalch** and **Lundsgaard's** results[1] these figures would imply a rise in the C_H. in the arterial blood of about $0{\cdot}0163 \times 10^{-7}$, and $0{\cdot}013 \times 10^{-7}$ respectively.

---

[1] *Biochem. Ztschr.* XXXVIII. p. 77. 1912: XLI. p. 247. 1912.

CONCLUSIONS.

1. During the hyperpnœa caused by breathing air containing CO_2 the dead space is considerably increased.

2. A rise of 2·5 mm. in the alveolar CO_2-pressure was found to be sufficient to cause a rise of 10 litres in the alveolar ventilation, and a rise of 2·0 mm. in the alveolar CO_2-pressure sufficient to cause a rise of 10 litres in the total ventilation of the lungs.

The expenses of this research have been in part defrayed by a grant from the Government Grant Committee of the Royal Society.

CEREBRO-SPINAL FLUID. III. THE GENERAL EFFECTS OF INCREASING THE CEREBRO-SPINAL PRESSURE[1]. By W. E. DIXON AND W. D. HALLIBURTON.

(*From the Physiological and Pharmacological Laboratories, King's College, London, and the Pharmacological Laboratory, Cambridge.*)

In our second paper we dealt with the effects produced by moderate changes in the cerebro-spinal pressure (C.S. pressure) on the arterial and venous pressures. In the present paper we deal with the effects produced by greatly increasing the C.S. pressure.

Several observers have described the effects of experimental cerebral compression. **François Franck**[2] observed the effects of compressing the brain in dogs and particularly noted respiratory paralysis, slowing of the heart-beat and elevation of arterial pressure. **Naunyn** and **Schreiber**[3] stated that the effects of cerebral compression are most marked when the compression exceeds the normal arterial pressure; they draw attention in particular to the rise of arterial blood-pressure and to the occurrence of Traube-Hering waves. The subject was also investigated by **Horsley** and **Spencer**[4] and later by **Horsley** and **Kramer**[5] mainly from the point of view of practical surgery. These investigators produced compression by inserting a bag into the cranial cavity through a trephine hole; this bag was distended with mercury which was run into it from a burette, so that the pressure applied could be accurately gauged by the height of the mercurial column: the effects of varying pressures on the circulatory and respiratory systems were then studied by the graphic method. They found that the primary cause of death is due to arrest of the respiration, and not to failure of the circulation. Respiratory failure is preceded by characteristic Cheyne-Stokes breathing, and is due to anæmia of the respiratory centre. This view of the cause of death was subsequently confirmed by **Leonard Hill**[1]. The matter is summed up thus by **Horsley**[2]:—

---

[1] The two preceding papers of this series appeared in this *Journal*, XLVII. p. 215. 1913, and XLVIII. p. 128. 1914.

[2] *Trav. du laboratoire de Marey*, III. p. 273. 1877.

[3] *Arch. exp. Path. Pharm.* XIV. 1882.

[4] *Phil. Trans. of the Roy. Soc.* CLXXXIII. B, p. 201. 1891.

[5] *Ibid.* CLXXXVIII. p. 223. 1897. See also **Horsley**. *Quart. Med. Journ.* July, 1894. These papers contain numerous references to the work of the older investigators.

"It would appear that of all the lower nerve centres which are necessary for the functions of so-called organic life, the respiratory centre is the most sensitive to mechanical pressure and shocks; that in cases when death is imminent, in cases of intracranial pressure, artificial respiration should be immediately performed and the skull opened freely; that in cases of sudden shock artificial respiration should be directly instituted."

Some years later **Harvey Cushing**[3] re-investigated the question, using much the same method of compression as **Horsley**. He devoted special attention to the rise of arterial pressure: and found that when the artificial compression exceeds the normal arterial blood-pressure, the latter rises and attains a level higher than that of the intracranial pressure. In this way the blood-pressure could be carried to "indefinite" heights (250 mm. of mercury or more) and the blood-pressure for a time overcomes the pressure applied to the brain, until at last paralysis of the vaso-motor centre ensues and the animal dies. The vaso-motor centre thus exercises a protective action, preventing the occurrence of anæmia in the bulbar centres. **Cushing** does not agree with **Horsley** and his colleagues on the primary importance of respiratory failure; he regards the cessation of breathing as the result of fatigue or failure of the vaso-motor centre which allows the blood-pressure to fall below the intracranial pressure. Anæmia of the respiratory centre consequently ensues, and respiration already embarrassed immediately ceases: at this stage, unless relief is given, the blood-pressure sinks to a level which represents irrecoverable paralysis of the respiratory centre. This difference of opinion is fundamental, and is of more than academic interest since it involves the practical question of the treatment of patients suffering from cerebral compression[4].

---

[1] *The Cerebral Circulation*, p. 187. 1896. London, Churchill & Co.

[2] *Quart. Med. Journ.* July, 1894.

[3] *Johns Hopkins Hosp. Bull.* XII. No. 126, Sept. 1901. *Amer. Journ. of Med. Sci.* CXXIV. No. 3, Sept. 1902. *Mitt. aus den Grenzgebieten d. Med. u. Chir.* IX. 1902. At the conclusion of this paper the editor (Naunyn) criticises the theory advanced.

[4] The question is also discussed from the clinical standpoint in cases of compression due to accumulation of cerebro-spinal fluid in meningitis etc. by **Parisot**, *Compt. rend. de la Soc. de Biol* LXVI. p. 939, 1909. **Cushing** deals with the clinical aspect in a later

In the present experiments it was found that the respiratory centre is always the first of the bulbar centres to fail, and this with a degree of compression which has no paralytic effect on the other centres. On cessation of a prolonged compression which has caused paralysis of all the centres, the respiratory centre is the last to recover; and unless artificial respiration is kept up during the compression the animal will die.

Our experiments were made on dogs fully anæsthetised with urethane and morphine, and the method we employed was simpler than that of Horsley and not altogether comparable with it. By means of the trocar and cannula already described the sub-cerebellar cisterna was punctured, and on withdrawal of the trocar the cannula is so firmly fixed that any tying of it in position is unnecessary, even although high pressures may be applied. The cannula is then attached to the lower end of a Woulff's bottle holding about 300 c.c. of Ringer's solution by a pressure tube filled with the same solution. Passing through the rubber cork of this bottle and communicating with the air space above the saline are two tubes, one attached to a mercurial manometer by which the pressure is read off and recorded, and the other to a bicycle pump by which the pressure can be increased. When the C.S. pressure is raised in this way by forcing Ringer's solution into the cranio-spinal cavity under pressure, considerable absorption of the fluid occurs; sometimes when the pressure is raised 300 mm. of mercury as much as 50 c.c. during the first minute may be absorbed, though absorption tends to become slower later: therefore if the increased pressure is to be kept constant the pump must be kept working.

With moderate degrees of compression such as 80 mm. of mercury, or higher pressures applied for brief periods of a few seconds, the effects recorded are clearly those produced by stimulation of the principal bulbar centres. The heart is slowed; this is evidently due to stimulation of the vagus centre, since section of the vagi removes the inhibition; but the arterial blood-pressure in spite of this inhibition either remains at its previous level, or, as is more common, rises above it; this is due to stimulation of the vaso-motor centre which counteracts or more than counteracts the fall of arterial pressure which simple cardiac inhibition would produce. The blood-vessels in the splanchnic area and limbs

---

paper, *Amer. Journ. of Med. Sci.* June, 1903, and quotes **Kocher's** articles:—**Hirnerschutterung** and **Hirndruck** in *Nothnagels Spec. Path. u. Ther.* IX. Th. 3, abth. 21, p. 186, in support of his view that cessation of breathing is a secondary result of vaso-motor failure.

become constricted and the constriction is greatly diminished in the splanchnic region by section of the splanchnic nerves. The respiration varies; it is sometimes a little accelerated, especially at first, but is more usually slowed; that is to say, even with these moderate pressures the effect on the respiratory centre is depressing rather than stimulating. On removing the pressure, well-marked Traube-Hering (Mayer) waves are seen on the tracing of arterial pressure, but the slow "vagus-heart" continues for some little time. By the previous administration of atropine the effect on the heart-rate can be eliminated and a rise of arterial pressure associated with Traube-Hering waves due to stimulation of the vaso-motor centre is then much better marked.

With greater degrees of compression other obvious effects are profuse salivation and dilatation of the pupil, both of which pass off when the compression ceases.

Fig. 1 represents a typical tracing of the arterial pressure when the artificial compression applied *via* the cerebro-spinal fluid was great (300 mm. of Hg), that is higher than the blood-pressure can overcome. As the blood-pressure begins to rise natural respiration ceases and artificial respiration must be started; if this be delayed even a little time the animal dies. The blood-pressure continues to rise in spite of the slow heart-beat until a stage is reached (generally suddenly) when the cardio-inhibitory centre becomes paralysed and the arterial pressure mounts still further due to the release of the heart from vagus control whilst the action of the vaso-motor centre is still unrestrained. Finally this centre is paralysed in its turn and the arterial pressure then gradually falls below the normal. At this time the pupils are widely dilated and salivation is profuse. If now the compression of the central nervous system is stopped, after a short delay the vaso-motor centre gradually recovers and the blood-pressure rises; at a later period recovery of the vagus centre takes place and the heart becomes slower; and still later the respiratory centre renews its activity. The rate at which the arterial blood-pressure rises after the compression has ceased depends primarily on the length of time and degree of constriction. After a compression of 400 mm. of Hg lasting from six to eight minutes which produces paralysis of the vaso-motor centre in the third minute, on removing the compression the rise in blood-pressure is very gradual for the first two or three minutes and then it becomes suddenly steeper and in the third minute it may rise 80 mm. of Hg. After each successive compression the rise after decompression becomes more and more delayed. After relatively short compressions such as those lasting four or five minutes, recovery is associated with very marked Traube-Hering waves but after the longer compressions these are less defined.

Fig. 1. Shows the effect on the arterial pressure of increasing the c.s. pressure to 300 mm. Hg. At *A* natural respiration ceased and artificial respiration commenced. At *B* the cardio-inhibitory centre became paralysed and at *C* the vaso-motor centre failed. The compression was continuous throughout the limits of the tracing. Time = secs.

If during a compression or immediately after it has ceased, strychnine is injected into the femoral vein, no convulsions are produced, showing that the spinal centres are also deeply depressed; these recover last of all, at least so far as the strychnine test can be relied upon. It is not uncommon to wait from ten to fifteen minutes after decompression before the onset of the strychnine convulsions occurs, at a time when all the medullary centres have regained their activity. At the time when the cord is irresponsive to strychnine, an intravenous injection of nicotine or adrenaline (drugs which act on peripheral and not central mechanisms) immediately produces the typical pressor effect. After decompression natural respiration always returns before the spinal centres (as tested by strychnine) recover. Hydrocyanic acid injected intravenously at a time when strychnine is inactive, produces its usual effect in accelerating and increasing the depth of respiratory movements owing to its action on the respiratory centre. If the motor-cells in the medulla and cord are paralysed by some drug such as apocodeine, then increasing the C.S. pressure produces no general vascular changes. To show this it is necessary to inject the apocodeine into the general circulation until nicotine ceases to exert a pressor action. Generally about 20 c.c. of a 1 °/₀ solution suffices. The animal must, of course, receive artificial respiration throughout.

Raising the C.S. pressure in this way exerts pressure upon the cerebral vessels, so that the blood-flow from the veins coming from the brain is either diminished or stopped. Thus in one experiment the blood-flow from the internal jugular vein was normally at the rate of 15 c.c. a minute; when the C.S. pressure was raised to 200 mm. Hg the flow from this vein was not appreciably altered, though when the pressure was raised to 400 mm. Hg the rate of flow diminished to 4 c.c. a minute; after the pressure was removed and whilst arterial blood-pressure was high the rate of flow returned quite suddenly to 18 c.c. a minute. In another experiment on a small dog, the effect on the blood-flow from the internal jugular vein caused by raising the C.S. pressure to 350 mm. Hg, was to reduce it from 5 to 1½ c.c. per minute. It is a general rule that when the rate of blood-flow from a vein is greatly diminished by a cerebral compression, that so soon as the maximum diminution is reached, which is also generally at a time before the blood-pressure has reached its maximum height, then the rate of flow tends to increase once more, although the C.S. pressure is kept constant.

If the rate of venous flow is taken from the torcular a rise of C.S. pressure from 4 mm. to 200 mm. in most experiments causes immediately a diminution in blood-flow, which however does not usually remain at this low level but tends to increase as the pressure is continued.

A rise of 400 mm. of Hg generally stops all venous circulation though cases are not wanting in which some flow of blood from the torcular cannula continues even with this pressure. In one experiment, under what we considered ideal conditions, the normal rate of flow from the torcular was 126 c.c. per minute. When the C.S. pressure was raised to 300 mm. Hg the flow registered 90 c.c. per minute; after an interval the pressure was raised again to 400 mm. Hg and the blood-flow now diminished to 78 c.c. per minute. A subsequent observation on the same animal showed that the rate of blood-flow from the cerebral end of the left internal jugular vein was 5 c.c. per minute, but that when the C.S. pressure was raised to 350 mm. Hg this diminished to 1½ c.c. per minute or less. This seems to suggest that in some animals the blood may regurgitate and that the torcular blood under these conditions may come from the right auricle.

It has been already pointed out that some flow from the internal jugular vein is the rule no matter what the pressure may be. The internal jugular vein however besides drawing blood from the base of the brain also receives blood from the pharyngeal venous plexus and occasionally from other parts, and this no doubt accounts for the fact that so long as the heart is beating no degree of compression entirely stops the blood-flow from this vein.

Attention has already been drawn in Part II to the effect upon the cerebral venous pressure of raising the C.S. pressure to a moderate extent. If the pressure is raised to anything from 100 to 400 mm. of Hg the changes are different and to some extent vary with the rate at which the pressure is raised so that different records may be obtained in different animals. Supposing the C.S. pressure is suddenly increased to 350 or 400 mm. of Hg and that the vagus centre is not very active, then arterial blood-pressure also quickly rises; that is, a decided rise may commence in twenty or thirty seconds whilst the venous pressure is affected immediately. Some initial rise of venous pressure is not uncommon and occurs at the moment the C.S. pressure is raised. If the C.S. pressure is raised very rapidly the smaller veins will be compressed first and then the larger, so that for a few seconds a greater flow of blood may pass along the larger veins and this might give rise to a temporary rise in venous pressure. So great an increase in the C.S. pressure as we are now considering is certain to exert some pressure on all cerebral vessels, and evidence of this has already been given in the diminished outflow of blood from the torcular and internal jugular vein during such augmented pressure.

Records of the torcular pressure show a small fall when the C.S. pressure is raised: sometimes this fall is preceded by a rise lasting a few seconds only. When the augmented C.S. pressure is reduced to the normal suddenly, at a time let us say when the arterial blood-pressure is high, the blood-flow from the veins increases at once and a considerable rise in venous pressure ensues which gradually resumes the normal level: this hump in the venous pressure is most readily interpreted by supposing it to be caused by the penned up blood behind being suddenly forced forward. Records of the venous pressure taken from the cerebral end of the internal jugular vein show curves roughly similar to those taken from the torcular. A curve of this character (Fig. 2) should be regarded then as typical of the venous pressure under these conditions. Thus in one experiment in an atropinised animal, increasing the C.S. pressure 300 mm. Hg caused an immediate rise of venous pressure from 10 to 25 mm. of Hg; this rise only lasted three or four seconds, after which the pressure rapidly sank to 4 mm. and remained at this level till the pressure was removed when it bounded up to 24 and then rose more slowly to 30, after which it sank gradually to normal. In this experiment the arterial blood-pressure rose from 120 to 220 mm. Hg, and it was at this maximum rise that the C.S. pressure was reduced. In this instance an increased C.S. pressure of 300 mm. did not block the flow of blood through the torcular opening though it greatly diminished it. It has been mentioned already that a high C.S. pressure may stop completely the flow of blood from the torcular cannula or it may reduce the flow only by a half or a quarter. For reasons already given we believe that this continued flow of blood may at all events in part be accounted for by blood coming backwards from the vessels heartwards.

Fig. 2. Shows the effect on the arterial pressure and torcular pressure in mm. Hg of increasing the C.S. pressure to 350 mm. Hg.

It sometimes occurs in trephining over the torcula that the cerebral wall of the vein is accidentally injured. Compression experiments under such circumstances show both a more pronounced initial rise and a more pronounced fall. In two instances we have found that increasing the C.S. pressure causes a considerable rise in torcular pressure and that although the former is kept constant the latter diminishes slowly. It is possible that the explanation of these cases is afforded by some block between the torcular and internal jugular vein which dams up the blood temporarily until the rising arterial pressure is able to overcome it (Fig. 3). In one case the torcular pressure remained up at its high level and showed no variations until the C.S. pressure was lowered, when it dropped to normal: this effect is not due to the high arterial pressure, since if the experiment is continued until the vaso-motor centre is paralysed and

Fig. 3. Shows the effect on the arterial and torcular pressures in mms. of mercury of increasing the c.s. pressure to 400 mm. Hg.

the blood-pressure is subnormal the venous pressure still remains constant. The pressure in this type of experiment has completely occluded both the arteries and veins. This condition is however unusual and we have not been able to repeat it.

Fig. 4 shows the various types of effects which we have seen on the torcular pressure by raising the C.S. pressure to 350 mm. Hg. *A* and *B* are the more usual effects, *D* is clearly a modification of *B*: the effect shown in *C* has been obtained only in one animal.

From these observations it seems to be quite clear that the pressure in the veins is entirely controlled by mechanical factors of which the important ones are the pressure of the blood in the arteries, and the facility with which the blood can escape through the veins.

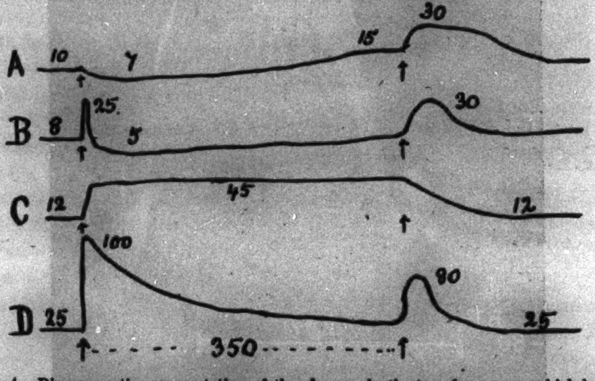


Fig. 4. Diagrammatic representation of the changes in the torcula pressure which have been observed during cerebral compression by raising the pressure in the c.s. fluid to about 350 mm. Hg. The length of each curve occupies about 5 mins.

SUMMARY.

1. A relatively small increase in C.S. pressure, artificially produced by a pump connected to the cranio-vertebral space by a cannula inserted into the subcerebellar cisterna, causes stimulation of the respiratory, vaso-motor and cardio-inhibitory centres. This stimulating effect is least marked on the respiratory centre.

2. When the pressure is increased to a height (300–400 mm. Hg) considerably above the normal arterial pressure, respiration ceases in a few seconds, but the other two centres remain active and the arterial

pressure rises enormously in spite of the slow heart; a little later the cardio-inhibitory centre is paralysed, and the continued activity of the vaso-motor centre still further raises the arterial pressure. This represents, as Cushing has pointed out, a protective mechanism which continues to force the blood along the compressed blood-vessels. Ultimately, however, if the high C.S. pressure is kept up, paralysis of the vaso-motor centre ensues, and the arterial blood-pressure falls. On removal of the compression, the centres recover in the reverse order, the vaso-motor centre first, and the respiratory centre last. The spinal centres as tested by the occurrence of convulsions after an injection of strychnine, recover their activity later still. Our results in relation to the early depression of the respiratory centre as the result of cerebral compression accord with the view of Horsley and his colleagues.

3. The changes in venous pressure during cerebral compression have also been studied; they vary in different animals and are not always easy to interpret.

We are indebted to the Government Grant Committee of the Royal Society and the Science Committee of the British Medical Association for grants towards the expenses of this research.

THE INFLUENCE OF A VITAMINE-FREE DIET ON THE CARBOHYDRATE METABOLISM. BY CASIMIR FUNK AND COUNT ERWIN v. SCHÖNBORN.

(*From the Department of Chemical Physiology, Cancer Hospital, Research Institute, London.*)

SOME time ago one of us[1] found that the addition of carbohydrates to a vitamine-free diet hastened the outbreak of beriberi. These experiments suggested that the addition of carbohydrates necessitates a greater use of the vitamine stock of the body and therefore rendered it probable that the vitamine plays an active part in the carbohydrate metabolism. The physiological role of the vitamines in the animal economy being unknown, it seemed advisable to extend the study in this direction and the present paper is a preliminary inquiry.

Pigeons were chosen for the experiments. We have determined both the normal glycogen content of the liver and the blood sugar on normal diet. To study the influence of the vitamine-free diet in varying proportions we have used five batches of pigeons which were fed artificially on different artificial food mixtures. The diet consisted of caseinogen, fat, sugar, starch and salts. The first diet was one containing all the ingredients, the second diet was free from sugar, the third free from fat, the fourth free from starch, and the last diet was entirely free from carbohydrates. The animals, all of which had the initial weight of 280 grms., were given these diets in the form of pills and were killed mostly after 14 days. The glycogen was estimated in the liver according to the usual methods and the blood sugar following the method of Michaelis and Rona and titration of the sugar following Bertrand's method. We shall see from the experiments that there was a marked disturbance of the carbohydrate metabolism, namely a high grade hyperglycæmia in cases of the diets containing a large proportion of carbohydrates. We have attempted in a few cases to correct this disturbance by an administration of vitamine from yeast. The number of the experiments are not sufficient to allow any definite statements to be made, but they tend to show that the addition of vitamine renders the carbohydrate metabolism more normal.

---

[1] Casimir Funk. *Ztsch. f. physiol. Chem.* LXXXIX. p. 378. 1914. *Ibid.* p. 378.

12·5 *gms. of the food mixture was given daily to each pigeon throughout all experiments.*

Pigeons on normal diet.

	Liver			Blood			
	Weight	Glycogen	%	Weight	Sugar	%	
1.	6·6	0·051	0·77	11·0	0·01	0·09	
2.	5·5	—*	—	12·0	0·01	0·08	
3.	7·3	0·051	0·70	14·1	0·014	0·10	
4.	7·5	0·102	1·33	—	—	—	
5.	5·2	0·121	2·3	12·1	0·017	0·14	
6.	7·9	0·061	0·77	12·7	0·013	0·11	Nematodes in liver.

* Glycogen estimation lost.

Exp. I. *Full artificial diet.*

omposition of the diet:—Caseinogen 12 p.c.
Starch 28 ,,
Fat 28 ,,
Sugar 28 ,,
Salts 4 ,,

	Liver			Blood			
	Weight	Glycogen	%	Weight	Sugar	%	
1.	5·5	0·039	0·71	9·5	0·017	0·18	Killed after 14 days.
2.	6·1	0	0	12·6	0·016	0·12	
3.	4·8	0·033	0·69	9·3	0·016	0·17	

Exp. II. *Carbohydrate-free diet.*

Diet—Caseinogen ... ... 12 p.c.
Fat ... ... 42 ,,
French chalk to make up pills 42 ,,
Salts ... ... 4 ,,

	Liver			Blood			
	Weight	Glycogen	%	Weight	Sugar	%	
1.	4·8	0·022	0·46	11·3	0·021	0·19	Killed after 14 days.
2.	4·0	0·011	0·27	9·5	0·025	0·26	
3.	4·7	0·011	0·25	12·0	0·022	0·18	

Exp. III. *Starch-free diet.*

Diet—Caseinogen 12 p.c.
Sugar 42 ,,
Fat 42 ,,
Salts 4 ,,

	Liver			Blood			
	Weight	Glycogen	%	Weight	Sugar	%	
1.	2·8	0·027	0·76	5·5	0·012	0·22	Killed after 14 days.
2.	3·5	0·010	0·29	9·5	0·020	0·21	
3.	2·7	0·027	1·0	11·3	0·022	0·20	

Exp. IV. *Fat-free diet.*

Diet—Caseinogen 12 p.c.
Sugar 42 ,,
Starch 42 ,,
Salts 4 ,,

	Liver			Blood			
	Weight	Glycogen	%	Weight	Sugar	%	
1.	5·8	0·325	5·6	10·9	0·010	0·09	Killed after 14 days. Nematodes in liver.
2.	7·1	0·255	3·6	—	—	—	
3.	10·1	0·950	9·4	—	—	—	
4.	3·0	0·027	0·9	8·0	0·012	0·15	
5.	3·5	0·078	2·2	8·3	0·018	0·21	

The same diet with addition of vitamine.

4·2	0·016	0·4	6·6	0·015	0·23

Exp. V. *Sugar-free diet.*

Diet—Caseinogen 12 p.c.
Starch 42 ,,
Fat 42 ,,
Salts 4 ,,

	Liver			Blood			
	Weight	Glycogen	%	Weight	Sugar	%	
1.	4·1	0	0	9·8	0·035	0·36	Killed after 14 days.
2.	3·7	0	0	11·5	0·035	0·30	
3.	4·5	0·006	0·01	11·0	0·029	0·26	
4.	4·6	0	0	6·3	0·015	0·24	Killed after 12 days.
5.	5·9	0	0	4·5	0·013	0·29	
6.	6·8	0	0	13·0	0·017	0·13	After 13 days had beriberi.

The last pigeon developed beriberi, the question whether the blood sugar diminishes in an attack of beriberi will be especially investigated.

The same diet with vitamine addition.

1.	4·7	0·033	0·7	11·6	0·023	0·20	12 days.
2.	4·4	0·022	0·5	8·7	0·017	0·19	13 days.
3.	4·9	0·033	0·68	11·4	0·021	0·13	

In all the experiments except those of Exp. V in which vitamine was given the weight of the animals dropped (in some cases by 20 %).

SUMMARY.

1. Pigeons kept on vitamine-free diet develop a tendency to hyperglycæmia with diminution of the glycogen of the liver.

2. This hyperglycæmia is especially marked in the case of sugar-free diet, which is followed by an entire disappearance of liver glycogen.

3. The addition of vitamine from yeast to the latter diet produces, in the three experiments made, a very marked formation of glycogen in the liver and a diminution of the sugar in the blood. Vitamine thus seems to have a distinct influence on the glycogen metabolism.

4. In case of the fat-free diet the glycogen content of the liver is increased.

ON SOME CARDIAC REFLEXES. BY F. A. BAINBRIDGE.

(*From the University of Durham College of Medicine.*)

BAYLISS(3) has demonstrated the existence of reciprocal innervation in vaso-motor reflexes, and it is often assumed that in reflex slowing or acceleration of the heart there is a similar balanced action between the vagus and the cardiac accelerators. The question does not appear, however, to have been directly studied in any of the numerous observations which have been made on cardiac reflexes, and the primary object of the present enquiry was to investigate it. This investigation has led to an analysis of the various factors concerned in reflex acceleration of the heart, and to the consideration of their general physiological significance.

Methods. Most of the experiments were carried out on dogs; cats and rabbits proved rather unsatisfactory mainly because of the comparatively slight vagus tone normally present in these animals. After a preliminary dose of morphia the dogs were anæsthetised with a mixture of chloroform and ether. A cannula was placed in the carotid artery; and one limb of the cannula was connected with a mercurial manometer which recorded the blood-pressure. The other limb of the cannula was attached to a Hürthle manometer which served to give a record of the pulse rate, since the reading of the mercurial manometer is often far from accurate. The respiratory movements were recorded either by means of a stethograph connected with a tambour, or by attaching the tambour to a side tube on the tracheal cannula. It is very important to avoid hæmorrhage, which partly by abolishing the tone of the vagus, partly by its general effects, renders it difficult and sometimes impossible to elicit the cardiac reflexes.

1. *Reflex slowing of the heart.* Although the production of reflex slowing of the heart by stimulation of the central end of one vagus and the disappearance of the reflex on section of the opposite vagus is so well established, the presence or absence of accelerator tone does not appear to have been specifically examined in such experiments. In the

present enquiry the existence of a marked accelerator tone was demonstrated by the fact that the pulse rate after section of the vagi was frequently almost maximal as was shown by the failure of adrenalin, when injected into the circulation, to produce any further acceleration. In other experiments in which the pulse rate after section of the vagi was not very rapid, the accelerator tone was increased by stimulation of the central end of the sciatic nerve; this, as will be shown later, reflexly stimulates the accelerator nerves, and often causes a prolonged increase in their tonic activity. Even when the accelerator tone was well marked stimulation of the central end of one vagus (after section of both vagi) had no effect whatever on the pulse rate. It seems clear, therefore, that the slowing of the heart normally produced by stimulation of the central end of the vagus or the lingual nerve, is due solely to reflex vagus inhibition and that there is no evidence for the existence of any associated inhibition of the accelerator tone.

2. *Reflex acceleration of the heart.* As is well known, stimulation of the central end of many sensory nerves reflexly quickens the heart and also causes hyperpnœa and usually a rise of blood-pressure. The exact time relations between the circulatory changes and the hyperpnœa do not appear, however, to have been recorded. Cyon and Roy and Adami (18) attributed the acceleration to reflex stimulation of the accelerator nerves. Hunt (11), whose paper contains a summary of the earlier literature, concluded that it was due entirely to diminution of vagus tone; he failed to obtain any reflex acceleration of the heart after section of the vagi. Mac William (14) also took the view that the acceleration was due solely to diminution of the tone of the vagus. Hooker (10), however, brought forward evidence that after section of the vagi reflex acceleration of the heart could be produced.

In the present enquiry the nerve used for stimulation was the central end of the sciatic trunk. A typical result is shown in Fig. 1.

The latent period of the acceleration and of the hyperpnœa is very short, usually not more than 1–2 seconds. Occasionally the rise of blood-pressure is replaced by a fall, the varying effects upon the blood-pressure being possibly due to variations in the depth of the anæsthesia. The acceleration is equally readily obtained in a curarised animal and is therefore independent of muscular and respiratory movements, as is seen in Fig. 2.

The possible causes of this acceleration are (1) diminution of vagus tone, (2) reflex stimulation of the accelerator nerves, and (3) the setting free of adrenalin. The chief share seems to be taken by the vagi, since

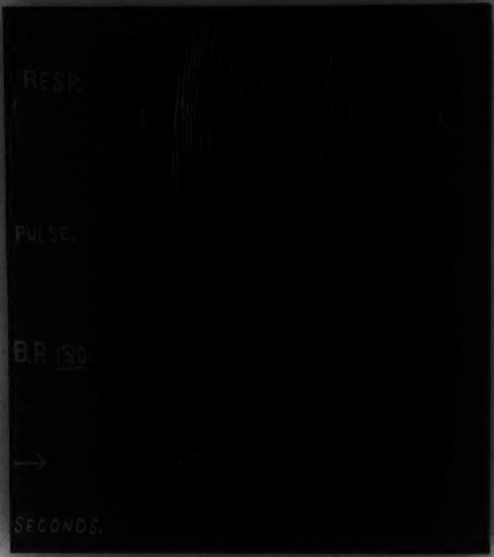


Fig. 1.

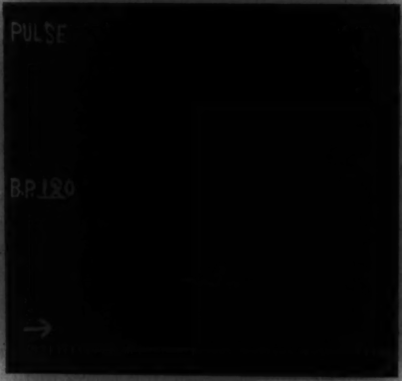


Fig. 2.

the acceleration is equally well shown after exclusion of the suprarenal glands from the circulation (Fig. 3), and stimulation of the central end of the sciatic nerve after removal of the stellate ganglia also causes an immediate definite acceleration (Fig. 4). Further the extremely short latent period shows that the initial effect at least cannot be due either to the accelerator nerves or to adrenalin.

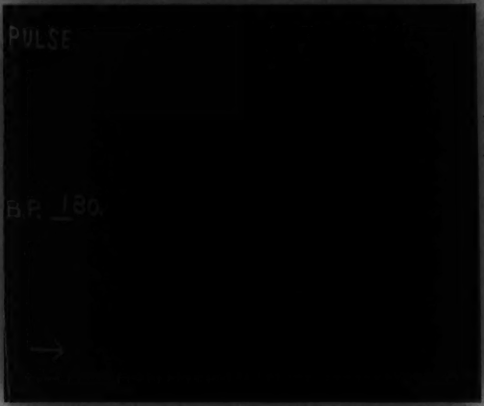


Fig. 3.

Fig. 4.

Diminution of the vagus tone, however, is not entirely responsible for the acceleration since this occurs even after section of both vagi, although it is often difficult to demonstrate owing to the rapid rate of the heart beat. The method employed by **Hooker** (10) for this purpose consisted in applying to the peripheral end of one vagus a current just strong enough to keep the heart beating at the rate previously observed in the intact animal. Intercurrent stimulation of the sciatic nerve then caused acceleration of the heart. This method has not been found entirely satisfactory in the present enquiry. The slightest escape of current alters the strength of the stimulus applied to the vagus and allows the heart to quicken; and the hyperpnœa accompanying the stimulation of the sciatic nerve makes it difficult to avoid such alterations in the strength of the vagal stimulus. The results thus obtained can only be regarded as reliable when the heart returns to its former rate after the cessation of the intercurrent stimulation (Fig. 5).

Fig. 5.

The vagus was being stimulated during the whole period shown in Fig. 5.

An alternative method is to slow the heart by means of a small dose of pilocarpin In some such experiments the respiratory system was not disturbed, and stimulation of the sciatic nerve led to a definite acceleration of the heart.

The most satisfactory results were obtained when, after section of the vagi, the heart-beat was not very rapid. The stimulation of the sciatic nerve then produced well-marked acceleration of the heart. It was found, however, that the acceleration tended to persist after the cessation of the stimulus, and that after the application of several stimuli the heart continued to beat almost, or quite, at its maximal rate, since the injection of adrenalin (1 c.c. of 0·05 p.c.) produced no further acceleration. This observation suggested that one factor in the acceleration of the heart was an increase in the tone of the accelerators which, as Hunt found, when it has once occurred tends to persist throughout the experiment. The fact that (Fig. 6) acceleration of the heart was produced after exclusion of the suprarenal glands, and section of the vagi shows that this suggestion is correct, since in these circumstances the only nervous path to the heart is the accelerator nerves, and in the curarised animal the influence of movement is excluded. It has not seemed necessary to investigate very carefully the part played by adrenalin in this reflex acceleration since the work of Elliott (8);

Fig. 6.

Fig. 7.

Cannon (8), **von Anrep** (16) and others has shown so clearly that any reflex rise of blood-pressure sets free adrenalin into the circulation. One or two experiments have been made, however, in which the stellate ganglia were removed and the vagi divided; stimulation of the sciatic nerve produced acceleration of the heart (Fig. 7) after a latent period of from 6 to 8 seconds. Owing to the length of the latent period a rather prolonged stimulation of the sciatic nerve is necessary.

DISCUSSION OF RESULTS.

The reflex acceleration of the heart which follows stimulation of a sensory nerve (the sciatic) is brought about partly by diminution of vagus tone, partly by stimulation of the accelerator nerve, and partly by the entrance of adrenalin into the circulation. The vagus is the most important of these factors, and when the stimulus is weak, it is the only one which can be demonstrated to be present. Its importance is indicated by the fact that the acceleration of the heart, provided the vagi are intact, is as pronounced after the exclusion of the suprarenal glands as in the intact animal, and is very well marked after excision of the stellate ganglia. The readiness with which the vagus centre responds to stimulation of a sensory nerve, such as the sciatic, would seem to make the accelerator nerves comparatively unimportant. **Gasser and Meek** (9) regard them as a subsidiary mechanism which can be called into use at times of stress, and the fact that their tone often increases as the general condition of the animal deteriorates may perhaps be regarded as favouring this view. Although stimulation of the sciatic nerve can cause a transient acceleration of the heart, the stimuli tend, if prolonged or repeated, to produce a more persistent quickening which is due to a permanent increase in the tone of the accelerator nerves. In this respect, the reciprocal innervation involved in reflex cardiac acceleration does not fall strictly into line with most of the other examples of reciprocal innervation.

Considered as a whole the effects on the circulation and respiration brought about by stimulation of the sciatic nerve show a striking similarity to those observed in muscular exercise; in each case there is an immediate acceleration of the heart, a rise of blood-pressure, and hyperpnœa. The nervous origin of the hyperpnœa has been demonstrated by **Krogh and Lindhard** (13), and the recent work of **Gasser and Meek** (9) shows that the increased pulse rate occurring at the beginning of exercise is due mainly to a diminution of vagus tone (as

Miss Buchanan (5), Aulo (1) and Johansson (12) had suggested), and to a smaller extent to stimulation of the accelerator nerves, and the setting free of adrenalin. This similarity appears to be more than a mere coincidence. As Sherrington has pointed out, the essential response to a nociceptive stimulus is muscular movement, and it is probable that the visceral reflexes which occur either in response to a nociceptive stimulus or at the onset of muscular exercise, form a group, acting together as a single reflex. This can be evoked either by impulses from the cerebral cortex, as in exercise, or by impulses from the periphery. In exercise the visceral changes cannot be separated from the muscular movements, whereas in the anæsthetised animal they can be evoked, apart from muscular movement, by stimulation of a sensory nerve, such as the sciatic.

CONCLUSIONS.

1. It is known that stimulation of the central end of one vagus causes slowing of the heart by way of the other vagus, and in the experiments hitherto described the effect ceased on section of the opposite vagus. In these experiments, however, it was not shown that accelerator tone existed. The experiments have been repeated, in this enquiry, when accelerator tone was present; in no case does stimulation of the central end of the vagus slow the heart by decreasing the accelerator tone. There does not appear therefore to be any reciprocal action between the vagus and accelerator centres.

2. It is known that stimulation of the central end of the sciatic nerve causes acceleration of the heart in the anæsthetised animal (with or without curare); but there is a difference of opinion as to the cause of the acceleration. In these experiments it has been found to be mainly due to a decrease of vagus tone, but acceleration can also be caused by increase of accelerator tone; and it is brought about by reciprocal action between the vagus and accelerator centres.

Further, some late increase in the rate of the heart is produced by the setting free of adrenalin.

3. The effects on the circulation and respiration of stimulation of the sciatic nerve and of muscular exercise are very similar. It is suggested that the reflexes concerned in producing these effects form a group, acting as a single reflex, which can be evoked by impulses starting either from the cerebral cortex, or from the peripheral nervous system.

REFERENCES.

(1) **Aulo.** *Skand. Arch. f. Physiol.* xxv. p. 347. 1911.
(2) **Bayliss.** This *Journal*, xiv. p. 313. 1893.
(3) **Bayliss.** *Proc. Roy. Soc.* B, lxxx. p. 339. 1908.
(4) **Barbèra.** *Arch. f. d. ges. Physiol.* lxviii. p. 444. 1897.
(5) **Buchanan.** *Trans. Oxford Jun. Scientific Club*, p. 351. 1909.
(6) **Cannon.** *Amer. Journ. Physiol.* xxxiii. p. 356. 1914.
(7) **Cyon.** *Arch. f. d. ges. Physiol.* lxx. p. 126. 1898.
(8) **Elliott.** This *Journal*, xlvi. p. 283. 1913.
(9) **Gasser and Meek.** *Amer. Journ. Physiol.* xxxiv. p. 48. 1914.
(10) **Hooker.** *Ibid.* xix. p. 417. 1907.
(11) **Hunt.** *Ibid.* ii. p. 395. 1899.
(12) **Johansson.** *Skand. Arch. f. Physiol.* v. p. 20. 1893.
(13) **Krogh and Lindhard.** This *Journal*, xlvii. p. 112. 1913.
(14) **Mac William.** *Proc. Roy. Soc.* liii. p. 464. 1893.
(15) **Roy and Adami.** *Phil. Trans. Roy. Soc.* B, clxxxiii. p. 254. 1892.
(16) **Von Anrep.** This *Journal*, xlv. p. 307. 1912.

THE DETERMINATION OF LACTIC ACID.

BY CHARLES G. L. WOLF.

(From the Institute for the Study of Animal Nutrition, School of Agriculture, Cambridge University.)

AMONG the difficulties encountered in the estimation of lactic acid, none has perhaps given more trouble than the extraction of the acid from the solution after proteins are removed. The coefficient of partition is so low that repeated extraction of the aqueous solution with large quantities of ether must be employed if the removal of the acid is to be in any sense complete. The methods[1] employed vary from those of Hopkins and Fletcher who shake the aqueous solution with a definite quantity of ether a definite number of times, to that of Embden, who employs the highly efficient Lind extractor exhausting the aqueous solution for thirty hours. Even with this method Embden advises a further treatment for ten hours to ascertain that the extraction is really complete.

The starting point of the present work was the wish to obtain a method of extraction which would give nearly quantitative results and the less time commencing than the method used by Embden. In the course of the work I have examined some of the more recent methods for the determination of lactic acid, for it has been evident, especially from the results of Embden, that lactic acid estimation by means of the zinc salt do not agree with those obtained by the oxidation process of v. Fürth.

The solution of lactic acid used throughout was prepared from ten kilos of fresh lean beef, and was repeatedly purified by means of the lead salt. It was practically free from β-oxybutyric acid. The determination of the content of the solution was made with a very accurately standardized solution of N/10 sodium hydroxide, using phenolphthalein as an indicator. The result so obtained was used as a standard for comparison. The carbonates of lead and zinc employed in the work were those of Kahlbaum. The zinc carbonate was carefully washed; comparison of washed and unwashed lead carbonate showed no differences in behaviour.

---

[1] The literature of the quantitative estimation of lactic acid is extensive. The recent papers of Mondschein, *Biochem. Ztschr.* XLII. pp. 91 and 105. 1912; Ishihara, *Ibid.* L. p. 468. 1913; and Yoshikawa, *Hoppe-Seyler's Ztschr. f. physiol. Chem.* LXXXVII. p. 382. 1913, who give the necessary references to the literature. See also Embden's article in *Hdb. der Biochem.* v. p. 1255. 1912, and Neuberg, *Der Harn*, pp. 245–254 and 1170–1172. 1911.

It was necessary to follow up all the individual steps in the final analysis separately and the results so obtained are given.

1. *The determination of lactic acid in an aqueous solution by means of the zinc salt.* 10·0 c.c. of a solution of sarcolactic acid containing 4·78 % as shown by titration were diluted with water and treated with an excess of zinc carbonate. The mixture was boiled gently. After filtering off the excess of zinc carbonate, the filtrate was evaporated to dryness on the water bath and heated in an air oven to 110°. The residue weighed 0·1600 and 0·1608 gram. These results are equivalent to 4·77 % and 4·74 % respectively or 99·3 % of the theoretical amount.

A similar experiment gave the following results:

A solution of sarcolactic acid 2·47 % by titration, digested with zinc carbonate.

Zinc lactate		% lactic acid	% zinO in zinc salt
0·1662		2·46	33·6
0·1662		2·46	—
0·1682		2·49	—
0·1662		2·46	—
0·1692		2·50	33·6
	Average	2·47	

We may therefore say that given a pure solution of lactic acid in water, the acid may be quantitatively estimated by digesting with an excess of pure zinc carbonate.

2. *The determination of lactic acid by oxidation with potassium permanganate in acid solution.* This method which has received considerable attention of late is the one elaborated by v. Fürth and his pupils, and has been used somewhat extensively by Embden. One's attention is drawn to the very considerable discrepancies which occur in Embden's work between the results as obtained by the zinc method and by oxidation.

I have devoted a great deal of time to this oxidation in the hope of making the method more nearly quantitative than it is, without however increasing the yield of aldehyde above 95 °/₀ of the theoretical amount. A typical set of results are the following: using 10 c.c. of a solution of sarcolactic acid 2·47 °/₀ by titration, the percentages of lactic acid as found by oxidation were 2·27; 2·28; 2·24; 2·22; 2·22; 2·23. These results fairly concordant among themselves yield as an average 94·6 °/₀ of the theoretical amount.

A further 10·0 c.c. of the solution gave a content of lactic acid of 2·46 °/₀ as estimated by the zinc salt. This salt was then oxidized and gave only 2·18 °/₀ of lactic acid.

It is obvious therefore that for results of the highest class, the method of estimation by weight of the zinc salt is superior.

In the hope that over oxidation of the acid might be prevented by further dilution of the permanganate, I have employed solutions more dilute than N/100. The results were not more favourable than with the last named concentration.

In an investigation of the effect of potassium bichromate and sulphuric acid on lactic acid, in connection with the combined estimation of β-oxybutyric acid and lactic acid, I have examined the effect of Shaffer's treatment as applied to lactic acid. Small and practically negligible amounts of lactic acid are attacked by bichromate and sulphuric acid as used by Shaffer.

3. *The behaviour of solutions of lactic acid on evaporation.* Having determined the accuracy of the two above mentioned methods for the ultimate analysis of lactic acid, the next point of importance was the behaviour of solutions of the acid on evaporation in the water bath and in a vacuum. It is known that solutions of lactic acid made alkaline and evaporated are partially decomposed, so that evaporation always takes place in a slightly acid medium. Observers are not however agreed as to the volatility of lactic acid when evaporated in the open[1].

My experiments have shown that it is unadvisable to concentrate solution of lactic acid in open dishes on the water bath. I have encountered losses as great as 30 milligrams in 160 when the solution containing the acid had a volume of 500–600 c.c. and this was concentrated to 20–30 c.c. Such losses do not occur if the solution is concentrated at a pressure of 20–30 millimeters. The use of the well-known double necked Claisen distilling flask is advisable. From this it would appear that all concentration of lactic acid solution should take place at as low a temperature as possible in a vacuum.

[1] Hart and Willaman. *Journ. Amer. Chem. Soc.* xxxv. p. 919. 1913.

4. *The extraction of lactic acid from aqueous solution.* When many determinations of lactic acid are required, the use of an efficient liquid extractor presents considerable difficulties, which can only be appreciated by those who have used them. It is conceded that in order to get an effectual extraction, the liquid must be in motion. This is done by means of a rotating stirrer as in the apparatus of Czellmanowitz or in the smaller apparatus of Lind. The latter apparatus has been used extensively by Embden. The extraction lasts at least 30 hours, and one ought to prolong the extraction a further ten hours to ensure its completeness. From the point of the time taken the process is not ideal, and I have sought for other methods. Two have been found which give practically quantitative results, but one, in my judgment, has distinct advantages.

Utilizing the results obtained by Black[1] in the determination of β-oxybutyric acid, a series of experiments was carried out in which the concentrated solution of lactic acid acidified with phosphoric acid was mixed with plaster of Paris. When the mixture was setting, it was constantly stirred, and finally when quite hard was broken into pieces about 3–4 mm. in diameter. The dry powder was put in an ordinary Soxhlet apparatus, and extracted with washed ether.

In three to four hours the extraction is quantitative. Owing to the presence of phosphoric acid, a relatively large quantity of plaster is required and for 30 c.c. of liquid an extractor holding 200 c.c. is necessary. I have therefore examined other absorbents and have found that ordinary blotting paper, or the paper used in all chemical laboratories for fat extraction in milk, leaves nothing to be desired.

In my first experiments, the acid solution was allowed to run on filter paper and to evaporate at room temperature. This was found to be unnecessary and in fact undesirable. It is better to run the solution on the paper, and while wet to roll it up and place it at once in the Soxhlet extractor. Even at room temperature a certain amount of loss of lactic acid takes place, when small amounts are distributed over a relatively large surface.

A single sheet of the standard Adams' paper is sufficient to absorb 10 c.c. of solution. It is quite easy to evaporate the solution to this volume in vacuo, and use a further 10 c.c. of water to remove the solution quantitatively from the flask. The washings are placed on a second paper. The two papers are easily contained in a 100 c.c. ordinary Soxhlet extractor.

---

[1] *Journ. Biol. Chem.* v. p. 207. 1908.

I have also used with equal success strips of Ford's white "80 pound" blotting paper, which was cut into strips 8 cm. by 35 cm. The paper was extracted with very diluted hot acetic acid, and washed free from acid with hot water. The saving in cost is not commensurate with the trouble involved.

In the use of both these papers I have observed the extraction of a small quantity of a gummy substance, which is fortunately quite insoluble in water. It is necessary therefore to filter the aqueous solution of the acid before treatment with lead carbonate. The eventual zinc lactate obtained in this way is quite white and crystalline.

5. *The removal of proteins from solution containing lactic acid.* The removal of proteins may be done in a variety of ways, either by simple coagulation as in the Mondschein method, by alcohol as in the Hopkins-Fletcher method, or with a mercuric salt, best exemplified by Schenk's reagent. I have used the mercuric reagent and have found it entirely satisfactory. The resulting filtrate is water white, and after the removal of the excess of mercury contains nothing but lactic acid, the salts and free hydrochloric acid.

That the process about to be described gives practically quantitative results is shown by the following tables. For the sake of comparison certain experiments made with the Lind extractor are given.

Sarcolactic acid added to a solution of blood albumin, lactic acid added 2·47 %.

Time of extraction from paper 4 hours.

Weight of zinc salt		% Lactic acid	
Evaporation in vacuo	Evaporation, Water bath	Evaporation in vacuo	Evaporation, Water bath
0·1678	0·1436	2·47	2·12
0·1658	0·1396	2·45	2·06
0·1660	—	2·46	—

Extraction of sarcolactic acid from a solution of egg albumin. Lactic acid 2·47 %.

Lind apparatus		Paper method	
Zinc salt	% Lactic acid	Zinc salt	% Lactic acid
0·1596	2·36	0·1692	2·50
0·1534	2·27	—	—

The extraction of lactic acid from blood gave equally satisfactory results. A known quantity of the acid was added to blood, and the proteins removed with Schenk's reagent, and extracted, using paper coils.

The following table gives the results:

	Zinc salt		% Lactic acid	
Hours	Wet	Dry	Wet	Dry
2	0·1680	0·1602	4·97	4·75
3½	0·1604		4·74	
	0·1603		4·74	
3	0·1580		4·67	
4	0·1658		4·79	

Acid by titration 4·78 %.

Zinc salt direct—0·1610—4·77 % lactic acid.
0·1603 4·74

The average for all determinations was 4·78 %.

In order to contrast the results obtained by the Hopkins-Fletcher method, two determinations were made. The results were in close agreement, but low. The amount in the solution as determined by titration was 4·78 %, 85·6 % of this amount was recovered.

Extraction by Hopkins-Fletcher method.

Zinc salt	% lactic acid
0·1401	4·14
0·1368	4·04

The low values agree with the statement of Ishihara[1] that a quantitative extraction of lactic acid by means of successive shakings is practically impossible.

The method which I propose for the estimation of lactic acid in blood and tissues is as follows:

The blood or tissue is placed in ten times its volume of cold 2 % hydrochloric acid, and after standing an equal volume of 5 % mercuric chloride is added. The mixture is allowed to stand at least 12 hours and is then filtered off in a Buchner funnel. Wash repeatedly with small quantities of Schenk's reagent (equal parts of 2 % hydrochloric acid and 5 % mercuric chloride). The filtrate which is quite free from colour is then treated with hydrogen sulphide, and the mercuric sulphide filtered off on the pump using a conical funnel. The sulphide is washed with hot water. If a Buchner funnel be used, small amounts of mercuric sulphide come through. The excess of hydrogen sulphide is removed from the filtrate with a rapid current of air. The solution is ready for evaporation in a vacuum. It is not neutralized. The concentration is effected in a Claisen flask at as low a pressure as possible, and when the solution is evaporated to about 10 c.c., the contents of the flask are poured carefully on an Adams' extraction paper. The remaining solution is washed quantitatively from the flask with very small quantities of water. The washings are delivered on a second strip. While wet the strips are rolled up and placed in an ordinary Soxhlet extractor, and extracted with washed ether for three to four hours. The extraction should be fairly rapid, and for heating we use a small electric stove. It is also advantageous to employ a Soxhlet apparatus with grounded joints. If corks be used they should be extracted with ether before employing them.

---

[1] Ishihara, *loc. cit.*

At the end of three to four hours the ether flask is disconnected and about 100 c.c. of water added to the ether. The ether is then distilled off and all lactic acid extracted from the paper is in the aqueous solution.

The solution is allowed to cool and filtered. In this way any gummy material extracted from the paper is quantitatively removed. The filtrate is treated with an excess of lead carbonate at 100° C. The excess of carbonate and other insoluble lead salts is removed by filtration when cold. The lead in the filtrate is removed by hydrogen sulphide, filtered off, and the hydrogen sulphide removed with air. The filtrate is treated with an excess of washed zinc carbonate at 100°, and the filtrate after removal of the excess of zinc carbonate evaporated to dryness in a weighed glass dish dried at 110° and weighed. The zinc lactate so obtained is white and of silky texture.

I wish to thank my assistant Mr U. A. R. Dudley for his invaluable help with this work.

ON THE CONSTANCY OF THE SYSTOLIC OUTPUT UNDER VARYING CONDITIONS. BY JOSEPH MARKWALDER AND E. H. STARLING.

(*From the Institute of Physiology, University College, London.*)

IN a recent paper (1) from this laboratory it was pointed out that with gradual increase in the arterial resistance, starting from a blood-pressure of 20–30 mm. Hg. and keeping the venous inflow constant, the output at first increases with increased pressure until a maximum output is reached. This output is maintained until a point is reached at which the resistance becomes too great for the heart to overcome, when the output falls off rapidly. The point of highest pressure, at which the maximum output was obtained, seemed in these experiments to lie near the average blood-pressure found normally in the animal, so that the heart apparently began to fail as soon as the blood-pressure rose above normal.

In some experiments which we published last year (2) we found that the flow through the coronary vessels was dependent on the arterial blood-pressure. With a small total output of the heart and a high arterial pressure the flow through the coronary vessels might therefore form a very considerable fraction of the total output from the left ventricle. When the output of the left ventricle is measured by measuring the blood flow through the resistance of the heart-lung apparatus, only that blood is measured which has passed the opening of the coronary vessels. The reading is therefore less than the total output of the left ventricle by the amount of blood flowing through the coronary circulation. It seemed to us possible that the falling off in the measured output, observed when the arterial pressure was raised much above the normal pressure, might be due to the increased fraction of the blood which escaped through the coronary vessels without flowing through the apparatus, and that, if this factor were taken into account, the constancy of the output from the heart under varying arterial resistance might apply within much wider limits than had been found by **Knowlton** and **Starling**. We have therefore carried out some experiments on the heart-lung preparation from the dog, in which simultaneous measurements were made of the output as measured by the flow through the apparatus, and of the coronary flow as measured by the outflow from a cannula in the coronary sinus. The general arrangement of the apparatus was similar to that already described in papers from this laboratory (1)(3). The cannulæ employed were however as wide as could be introduced into the vessels, and the rubber and glass tubes of the apparatus were wider than had been previously employed. The blood flowing through the resistance passed through a wide glass spiral immersed in warm water and then discharged into a large venous reservoir which was connected with the cannula in the superior vena cava by a wide rubber tube. The reservoir was about 35 cms. above the level of the heart. The inflow of blood into the heart was regulated by a screw clip on the rubber tube passing from the venous reservoir. The systolic output was measured on the venous side of the resistance by allowing the blood to flow through a tube into a measuring cylinder, the time taken to collect 100 c.c. being determined by means of a stopwatch. The coronary flow was measured by collecting the blood flowing from the cannula in the coronary sinus in a graduated cylinder, and the time necessary to collect 20 c.c. was determined. The arterial pressure was measured by connecting the cannula in the innominate artery by means of a side branch with a mercurial manometer, the oscillations of which were recorded on a kymograph. The pressure in the inferior vena cava was measured by means of a water manometer connected with a long cannula inserted in the inferior cava in the abdomen and pushed up so that its orifice lay close to the entrance of the inferior cava into the right auricle.

It must be remembered that the flow from the coronary sinus only represents a portion (about $\frac{3}{5}$) of the total flow through the coronary circulation. To obtain the total amount of blood escaping from the aorta through the coronary arteries we must therefore multiply the figure obtained in this way by $\frac{5}{3}$. The results of two experiments obtained in this way are given in the following tables, and are represented graphically in the figures.

Certain points must be taken into consideration when discussing the results of these experiments. The venous inflow employed, and therefore the total output of the ventricles, is considerably larger than in the experiments recorded in **Knowlton** and **Starling's** paper. It is impossible to judge from the heart-lung preparation what is the

TABLE I. *Dog.* 10·75 *kilos.* *Weight of heart* 104 *gms.*

	Time	Arterial pressure mm. Hg.	Rate of heart per min.	Systemic output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary output (calculated)	Total output	Output per heart beat c.c.	Temperature	Venous pressure c.m. H_2O
I *a*	2.45	92		250	19·58	32·6	282·6	2·46	34·5	6·4— 7·2
	2.50	122	Heart irregular	248	29·85	49·7	297·7	2·5	35	6·0— 6·8
	2.54	170		224	50·42	84·0	308·0	2·58	35	5·2— 6·4
	2.58	208		196	85·72	142·9	338·9	2·6	35·5	5·2— 6·4
	3.2	164		202·6	61·86	103·1	305·7	2·35	35·5	5·2— 6·0
	3.5	126		202·6	40·54	67·5	270·1	2·07	35·5	5·6— 6·8
	3.8	78		227	20·76	34·6	261·6	2·0	35·5	6·0— 7·2
	3.11	46	120	238	13·43	22·4	260·4	2·16	35	6·0— 7·2
I *b*	3.15	64	120	1086	26·80	44·6	1130·6	9·45	35	10·4—12·0
	3.20	100	120	926·5	39·48	65·8	992·3	8·25	35	10·0—11·6
	3.23	140	120	910	52·37	87·3	997·3	8·3	35	10·0—11·6
	3.26	184	120	825	130·50	217·5	1042·5	8·7	35	10·0—11·2
	3.29	65	114	1000	34·29	57·1	1057·1	9·25	34·6	10·4—12·8
I *c*	3.34	112	114	2149	107·20	178·6	2327·6	20·0	34·6	18·8—20·8
	3·36	Commencing heart failure.							34·6	16·6—18·4
	3.39	1 c.c. adrenalin 1/10,000.								
	3.40	112	198	2143	166·70	277·8	2420·8	12·2	35	12·0—14·0
	3·43	144	210	2000	166·70	277·8	2277·8	10·8	35	12·5—15·0

Notes. Between *a* and *b*, and *b* and *c*, the venous inflow was increased.

TABLE II. *Dog.* 11 *kilos.* *Weight of heart* 107 *gms.*

	Time	Arterial pressure mm. Hg.	Rate of heart per min.	Systemic output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary output (calculated)	Total output	Output per heart beat c.c.	Temperature	Venous pressure cm. H_2O
II *a*	12.5	84	150	811	24·50	40·80	851·80	5·72	35·4	9·6—12·4
	12.8	108	150	790	31·90	50·30	840·30	5·60	35·3	8·8—12·0
	12.12	140	150	770	46·50	70·75	840·75	5·60	35·4	8·0—11·2
	12.15	170	150	750	71·43	117·40	867·40	5·75	35·5	8·0—10·8
	12.18	208	150	600	157·90	260·30	860·30	5·72	35·5	12·0—22·0
	12.21	104	150	760	49·60	80·30	840·00	5·60	35·5	8·4—10·4
	12.27	104	150	750	49·60	80·30	830·00	5·55	35·5	8·0—10·0
	12.30	44	150	790	20·40	30·40	820·00	5·45	35·5	7·6—10·0
II *b*	12.34	60	150	1402	31·90	50·30	1452·00	9·70	36·0	10·4—12·8
	12.38	100	150	1260	54·05	90·00	1350·00	9·00	35·8	10·0—12·8
	12.41	128	150	1222	76·92	124·90	1346·90	9·00	35·8	10·4—12·4
	12.45	166	144	1178	125·00	208·00	1386·00	9·65	35·5	12·0—14·0
	12.50	190	144	710	300·00	500·00	1310·00	9·20	35·5	32·0—34·0

Between II *a* and II *b* venous inflow increased.

output of a given heart. All we can judge of is as to the functional capacity of the heart. The estimations of the output of a dog's heart from cardiometer records are unsatisfactory as an index of the normal output in consequence of the abnormal conditions of an animal anæsthetised and with the chest widely open. According to **Krogh** (4) the extreme variations of the systolic output in man would be 3 litres and 21 litres per minute. In a dog of 11 kilos we may perhaps therefore regard similar limits as 500 c.c. and 3½ litres per minute. In the first experiment therefore we began with an output which is probably less than that existing in the resting animal, though perhaps not smaller than the amount which would occur during sleep. The figure of 3½ litres would only be expected for short periods of time and during active exercise.

It must be remembered too that the figure for the total coronary output is a calculated one on the assumption that the measured flow from the coronary sinus was ⅗ of the total. The exact proportion, as has been already shown (**Evans and Starling** (5)), varies from heart to heart, and it is evident that any error due to our employing the factor of ⅗ to multiply the coronary sinus output would be more marked the smaller the total output of the heart was.

The general results of these experiments will be evident on reference to Fig. 1, showing diagrammatically the results of the two experiments of which the numerical results have been given. In this figure, 1 and 2 refer to the two different experiments; 1 A and 2 A refer to the experiments with a moderate inflow of blood, and 1 B and 2 B to the experiments with a large inflow of blood. The total output, *i.e.* systemic *plus* coronary, in each case is represented by a thick line. The separate systemic and coronary outputs in one experiment are also represented by thin lines (2 B), and the venous pressure in the same experiment, as measured in the inferior vena cava, by a dotted line. The divisions along the abscissæ correspond to millimetres of mercury, and those along the ordinates correspond to the output in 100 c.c. per minute or to the venous pressure in mms. of water.

It is evident from reference to this diagram that, given a constant venous inflow, the total ventricular output is within very wide limits independent of the arterial resistance. Thus in Exp. 1 A the total output was 260 c.c. at 46 mm. Hg., 270 c.c. at 126 mm. Hg., 338 c.c. at 208 mm. Hg. We are inclined to regard this increase with increase of pressure as probably largely apparent and as indicating that in this particular animal our factor ⅗ is rather too large. On the other hand, there may be a certain degree of increased efficiency of the cardiac muscle associated with a rise of arterial pressure and the largely increased flow through the coronary vessels thereby induced. In the second experiment (2 A) the agreement between the outputs obtained is still more striking, namely, 820 c.c. at 44 mm. Hg., 851 c.c. at 84 mm. Hg., 840 c.c. at 140 mm. Hg., 860 c.c. at 208 mm. Hg. With a still larger flow (2 B) the output was also very constant and varied from 1452 c.c. at 60 mm. Hg. to 1386 c.c. at 166 mm. Hg. In this last experiment (2 B) we are evidently approaching the limit at which this particular heart could work over a considerable period of time. It was putting out about 1½ litres per minute, and it is worth noting the relation between the systemic output and the coronary output. With the rise of pressure the systemic output drops steadily from 1450 c.c. at 60 mm. Hg. to 1178 c.c. at 166 mm. Hg. On further increasing the arterial pressure to 190 mm. Hg. the systemic output fell markedly to 710 c.c. This was not associated however with a corresponding fall of

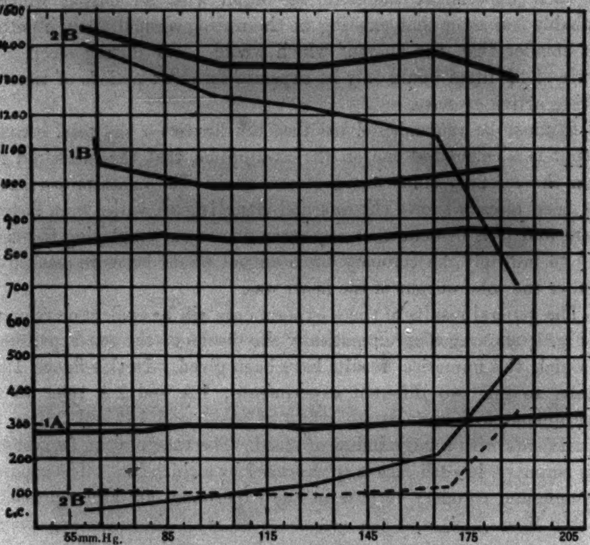


Fig. 1. Diagram showing total output of left ventricle with different ratio of inflow and varying arterial resistance.

the total output of the ventricle since it was nearly compensated for by a corresponding increase in the coronary flow. Thus the coronary flow between 60 mm. Hg. and 166 mm. Hg. rose from 50 to 208 c.c. per minute, and the further rise to 190 mm. Hg. more than doubled the coronary flow, which rose to the high figure of 500 c.c. per minute. This rise of coronary flow is out of all proportion to the rise in arterial pressure, and it is evident that some new factor must be added to the purely mechanical factor of blood-pressure to determine this increase. In our previous paper (2) we showed that the most effective agent in causing dilatation of the coronary vessels was non-gaseous metabolites produced by the heart muscle itself, and that these were produced in greater quantity the more unfavourable the conditions under which the heart muscle was contracting. We therefore ascribe this great increase in the coronary flow to the overstrain of the heart muscle represented by its effort to force out a litre and a half of blood a minute against a pressure of 190 mm. Hg. By means of this dilator mechanism the heart muscle is enabled to maintain an adequate circulation in spite of the great demands that are thrown upon it. This condition could not however endure for long without failure at some point of the cardiac mechanism, and the beginning of failure is shown by reference to the curve of venous pressure, also indicated by dotted lines in the diagram (Fig. 1). The venous pressures with low and moderate inflow are not recorded in this diagram since they remained constant and were unaffected by the changes in the arterial resistance. In Exp. II *b* with a large inflow and output, the pressure in the inferior cava increased from 104 to 120 mm. H_2O with a rise of arterial pressure from 60 to 160 mm. Hg. As the pressure was then put up from 166 to 190 mm. Hg., the venous pressure rose to 320 mm. H_2O. It must be remembered that we are measuring the outflow from the left ventricle and that we are increasing the resistance to the outflow from the left ventricle. The venous pressure is however measured in the vein running into the right auricle. A rise of pressure in the inferior cava therefore indicates (1) increased pressure in the left ventricle during diastole, *i.e.*, inadequate emptying of the left ventricle, (2) a rise of resistance to the contraction and outflow from the right ventricle, (3) incomplete emptying of the right ventricle and therefore increased diastolic pressure in this cavity and the adjoining vessels. It is worthy of note that the rise of venous pressure is noticeable before any considerable diminution occurs in the amount of blood put out by the ventricles against the high arterial resistance. The last thing to fail is the mechanical performance of the heart in the driving out of blood. Temporary heart failure is shown still more markedly at the end of Exp. 1. In this case with an inflow of 2½ litres a minute the heart could not contract efficiently against a pressure of 112 mm. Hg., the pressure ran up rapidly from 100 to 180 mm. H_2O and the heart began to drop beats. The administration of adrenalin improved the functional capacity of the heart and enabled it to empty its cavities at each contraction, so that the output rose to the full 2½ litres a minute and the venous pressure fell to 120 mm. H_2O.

The influence of temperature on the ventricular output. In our previous paper we mention that the flow through the coronary vessels varied very little with alteration in temperature, being somewhat greater at a low temperature. In view of the extreme susceptibility of the coronary vessels to the action of locally produced metabolites, it seems possible that this dilatation at low temperatures may be due to a slowing of the processes of oxidation, so that partly oxidised metabolites may escape from the cells and exercise a dilating influence on the coronary vessels. In the following experiment the total output (*i.e.* systemic *plus* coronary) was measured at varying temperatures and therefore at varying rates of the heart, first with an inflow of about 650 c.c. per minute and secondly with an inflow of about 1300 c.c. per min. The results are given in tabular form (Table III) and in a diagrammatic form in Fig. 2.

In this figure the total output, *i.e.*, systemic *plus* coronary, is represented as before by thick lines, A referring to the experiment with

TABLE III. *Dog.* 8·5 *kilos.* *Weight of heart* 77·5 *gms.*

	Time	Temperature	Rate of heart per min.	Arterial pressure mm. Hg.	Systemic output c.c. per min.	Coronary sinus output c.c. per min.	Total coronary output (calculated)	Total output (calculated)	Output per heart beat c.c.	Venous pressure cm. H_2O
III *a*	2.20	28·2	72	119	612	24·20	40·3	652·3	9·05	11·0
	2.28	30·2	90	119	612	20·62	34·4	646·4	7·20	9·0
	2.37	33·4	114	119	612	18·46	30·8	642·8	5·63	6·2
	2.42	35·0	126	119	625	19·74	32·9	657·9	5·2	5·0
	2.50	37·0	144	117	612	21·20	35·3	647·3	4·47	4·2
	2.58	39·0	156	116	612	22·90	38·1	650·1	4·16	3·8
III *b*	3	39·0	156	126	1250	35·72	59·5	1309·5	8·45	7·4
	3.5	37·0	132	126	1250	37·27	62·1	1312·1	10·00	7·8
	3.10	35·0	120	126	1250	36·15	60·2	1310·2	11·00	9·0
	3.14	32·8	102	126	1250	36·59	60·9	1310·9	12·95	11·0
	3.22	30·1	84	126	1200	44·14	73·6	1273·6	15·2	14·6
	3.30	28·0	72	126	1130	51·72	86·2	1216·2	16·9	16·2
	3.38	26·2	60	126	1030	98·36	163·9	1193·9	19·9	19·0

moderate inflow, about 600 c.c. per minute, and B to the experiment in which the inflow was doubled to about 1300 c.c. per min. In the case of the larger output the systemic output is also represented by a thin line, the vertical distance between the thin and the thick lines of the experiment (B) being equal to the coronary flow. It is evident from this diagram that within very wide limits, *i.e.*, from 26° to 39° C. the output of the heart, as stated previously by **Knowlton and Starling**, is practically independent of the temperature.

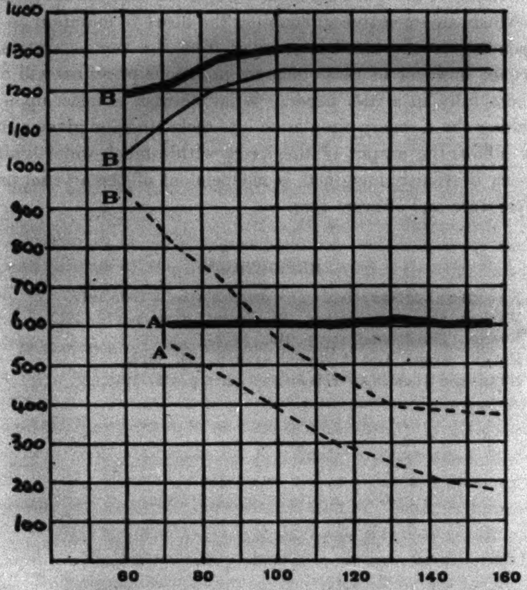


Fig. 2. Diagram showing influence of rate of heart beat (as determined by temperature on total output and coronary circulation).

Coordinates = c.c. output per min.
Abscissa = pulse rate per min.

Since in this experiment the rate of the heart beat varied with the temperature from 60 to 156 per minute, we may say that alteration in the rate of the heart beat, provided that the venous inflow is constant, has little influence in altering the output. This statement applies absolutely for the smaller inflow, at which the rates varied from 72 to

156. With a larger inflow there was a slight decrease in the output as the temperature fell from 32·8° to 26·2° C. with a diminution in the heart rate from 102 to 60. The changes in venous pressure with the changes in the rate of the heart are worth noting. They are especially marked in the case of the larger venous inflow when the heart was beating slowly at 60 per minute. It is evident that here, as in the case of increased mechanical demands on the heart muscle, the venous pressure rises before there is any falling off in the total output of the heart, so that the rise of venous pressure must be regarded as one of the mechanical means which are operative in enabling the heart to maintain an output corresponding to the blood it receives from the venous system. The significance however of these changes in venous pressures will be dealt with more fully in a later paper. What we wish to insist upon now is that, taking the coronary circulation into reckoning and given a constant venous inflow, the output of the heart, within much wider limits than have been previously imagined, is independent of the arterial pressure, of temperature and of heart rate.

REFERENCES.

(1) **Knowlton and Starling.** This Journal, XLIV. p. 206. 1912.
(2) **Markwalder and Starling.** This Journal, XLVII. p. 275. 1913.
(3) **Evans.** This Journal, XLV. p. 213. 1912.
(4) **Krogh.** Skand. Arch. f. Physiol. XXVII. S. 100. 1912.
(5) **Evans and Starling.** This Journal, XLVI. p. 413. 1913.

ON THE MECHANICAL FACTORS WHICH DETERMINE THE OUTPUT OF THE VENTRICLES.

By S. W. PATTERSON, *Beit Memorial Research Fellow*, AND E. H. STARLING.

(*From the Institute of Physiology, University College, London.*)

THE working capacity of a pump is measured by its output. In the heart the output has to undergo large alterations with varying conditions of the body, and its measurement is therefore the most important quantitative determination connected with this organ. Most of the previous researches on this subject have been carried out on the whole animal, and in view of the difficulty of controlling the different factors involved under these circumstances it is not surprising that considerable discrepancies exist between the views of different workers as to the relative importance of the various factors involved. Thus whereas Zuntz (1) and his pupils describe great variations in the output associated with exercise, and Krogh (2) has actually measured these variations in man, Yandell Henderson (3), as a result of a number of careful experiments, considers it impossible for the heart to more than double its output under any circumstances, though Krogh finds variations in the output of the left ventricle between 2·8 and 21 litres per minute. It seemed to us that the whole question might be put on a securer basis if investigated on the heart-lung preparation as used in this laboratory, where all the mechanical factors of the circulation are under strict control. The results obtained in this way should give us some idea of the capabilities of the heart as a pump, which might serve to explain the behaviour of this organ in the intact animal. It has been shown lately by Markwalder (4) and one of us that, provided the venous inflow be maintained constant, the output of the left ventricle is within wide limits independent of the arterial resistance, the small falling off in the output with rise of pressure, observed in previous experiments, being fully accounted for by the increased leakage from the aorta through the coronary system of vessels as the arterial pressure rises. Knowlton and **Starling**(8) found the output practically constant with varying rates of beat caused by differences of temperature, and this has been confirmed by **Markwalder** and **Starling**. On the other hand, the output is immediately increased by raising the venous reservoir or increasing in any way the inflow into the right heart. We must therefore focus our attention on the connection between venous inflow, venous pressure and ventricular output.

At the beginning of ventricular systole the ventricular muscle passes rapidly from a condition of relaxation to one of contraction. The auriculo-ventricular valves close at the very commencement of the ventricular contraction. The contraction of the muscle, or rather the rise of contractile stress, then proceeds for a time isometrically, while the pressure in the ventricle is rising to the pressure in the aorta. When the pressure rises to this point the aortic valves open and the muscle fibres are able to shorten. The pressure, however, continues to rise for a time and then falls, or it may run almost horizontally (the 'plateau'). During the plateau the contraction of the ventricular muscle is more isotonic. The extent to which the contraction differs from pure isotonic contraction will vary according to the height of the arterial resistance and the amount of blood which is being forced out of the ventricle. The more marked the 'plateau,' the more perfect will be the isotonicity[1]. The contraction then ceases and relaxation begins with coincident rapid fall of pressure in the ventricle below that in the aorta, which causes a closure of the aortic valves. From this time forward the relaxation of the ventricular muscle differs in its conditions from the relaxation of a muscle recovering from a muscle twitch. A skeletal muscle has no power to lengthen itself actively,—it has to be lengthened by the application of some extending force. It presents, however, little resistance to extension, so the extending force may be very small. On the other hand, the contractile state does not disappear instantaneously, so that the curve of lengthening of a muscle alters with variations in the extending weight, the lengthening period being shortened by increase of the weight on the muscle. In the same way, in order that the ventricular muscle may lengthen, the ventricles have to be distended by the inflow of fluid, though the pressure necessary to distend them is almost negligible. But whereas in the case of the skeletal muscle (under ordinary experimental conditions) the tension exerted by the extending force is the only factor of importance, in the heart the rate of application of the extending force, *i.e.* the rate of inflow of the blood, is still more important than the tension exerted by it on the walls of the ventricle. The ventricles cannot dilate unless fluid enters them, so that they may be, so to speak, 'after-loaded' at every stage of their relaxation. If blood therefore does not enter the heart, the ventricles do not dilate at all during diastole and may become smaller and smaller with each systole until they are empty. This condition has been described by Henderson, as the result of acapnia, as 'increased tonicity of the heart,' but it is purely a mechanical result of absent or deficient venous inflow. An 'adequate' venous inflow might therefore be defined as one which is exercising a minimal distending force on the ventricular muscle throughout the whole of its relaxation, so that at no time can the muscle be regarded as after-loaded. Under these circumstances, if the diastole be prolonged, the heart will dilate to its maximum or until restrained by the surrounding pericardium. As a rule, however, before this condition of extreme dilatation is attained, a new systole begins, the auricle empties itself into the ventricle, and then the ventricle contracts once again. If the venous inflow is still greater, *i.e.* more than adequate, it may exert an active tension on the ventricular muscle during its period of recovery from the contracted condition and during the whole of its subsequent relaxed condition. In this case the important factor is now the pressure at which the blood enters the auricles and ventricles. The higher the pressure the more rapidly it will stretch these structures, and the more rapid therefore will be the acquisition by the heart of its maximal condition of dilatation.

---

[1] In view of the complicated arrangement of the fibres of the mammalian heart, it is impossible to determine definitely the relation between the tension on each strand of muscle fibres or on each muscle fibril and the intracardiac pressure. If the cavity of each ventricle were perfectly spherical the tension on each muscle fibre would be greater the greater the capacity of the sphere, assuming the intracardiac pressure to remain constant. (The total pressure on the whole of the boundary layer of a sphere will vary as its surface and therefore as the square of the radius. While the length of each muscle fibre lying in the circumference of the sphere would vary directly as the radius, the amount of fluid to be forced out by a given shortening of the muscle fibres would vary as the cube of the radius.) Since under normal circumstances the capacity of the heart cavities becomes less during the contraction of the ventricle, a constant tension on the muscle fibres would tend to produce a pressure in the heart cavities which would increase steadily as the emptying proceeded and their capacity became smaller. If this were the case, a plateau, *i.e.* a period of constant pressure in the ventricular cavities, would be associated with a gradually diminishing load off the individual muscle fibres as they contracted, a condition which would be favourable for obtaining the maximum mechanical performance out of the contracting muscle. It may be remembered that under normal conditions in the body, most skeletal muscles contract in this way, since they act at a greater mechanical advantage or smaller mechanical disadvantage when contracted than when commencing their contraction. This condition is imitated in Fick's cosine lever. See **Burdon Sanderson**'s article in *Schäfer's Textbook of Physiology*, II. p. 355.

In view of these facts it is difficult to see how it is possible to maintain Henderson's dictum that the curve of filling of the heart is the same under all circumstances. The rate of filling must depend first on the rate at which the blood enters the heart from the veins, and secondly on the pressure at which the blood enters the heart. It will therefore be necessary to enquire into the influence of the variations of the venous pressure and venous inflow on the diastolic filling and the output of the ventricles. (The question of the volume curve will be dealt with in a subsequent paper.)

In dealing with variations of venous pressure, *i.e.* the pressure in the big veins at their entry into the auricles, it is important to remember that this may be altered under two conditions. In the first place with a moderate inflow, *i.e.* any amount up to what we have defined as adequate, variations in the rate of inflow of blood will first cause very little change of pressure, the increasing inflow being merely sufficient to relieve the after-loading of the ventricular muscle during its relaxation. As soon as the inflow exceeds the rate at which it can keep pace with the relaxation of the ventricular muscle, the pressure at the auricular orifice of the great veins must begin to rise, and this pressure will exert an active distending influence on the ventricular walls, so still further quickening the rate of filling of their cavities and increasing the output from the ventricles. On the other hand the venous flow must be maintained by means of a reservoir which is some distance above the level of the heart. If for any reason the heart fails in its pumping action, the blood will remain in the auricles and ventricles and will prevent the further entry of blood from the reservoir. The heart will dilate more and more after each inefficient systole, while the pressure in the great veins will rise steadily: but this rise of pressure will be associated not with increased output from the ventricles but with diminished output. We have therefore to distinguish between a rise of venous pressure which is effective in dilating the heart during diastole and increasing its output, and a rise of venous pressure which is passively induced by failure of the pumping action of the heart.

Under normal conditions, *i.e.* with a heart working under a load which it can easily accomplish, the pulmonary circulation introduces no complications. Commencing failure of the heart may, however, be marked either on the right or left side, and we shall have later to consider how the circulatory conditions in the lungs affect and are affected by the conditions in the systemic circulation.

In our experimental investigation of these points we have been met by several difficulties and experimental limitations, the existence of which in our early experiments we did not suspect. The first was due to our failure to appreciate the viscosity of the blood and the influence of this factor on its flow through tubes. We found that, in consequence of this viscosity, our venous reservoir with its system of fairly wide tubes was only delivering fluid at the rate of a few 100 c.c. per minute into the heart, and that the maximum output of the heart was really limited by the amount which would flow from our venous reservoir. It seemed easy to get over this by putting the venous reservoir higher and connecting it by a long tube with a cannula in the superior vena cava. This gave a larger inflow but had the disadvantage that if for a short time the action of the heart failed, the blood accumulated in the right heart at an enormous pressure which caused damage to the heart wall, or if the right heart responded fairly well, might cause pulmonary œdema. The same drawback attended our efforts to increase the flow into the right heart by exerting pressure upon the blood in the venous reservoir by blowing air into this vessel. These difficulties were finally obviated by using a large reservoir a short distance above the heart and connecting it with the venous cannula by a short piece of hose pipe, the rate of blood flow being regulated by a screw clip on the hose pipe. We met with the same type of difficulty on the arterial side of the system when attempting to determine the maximum output the heart was capable of. All our tubes were found to be too narrow, so that finally we had to insert a wide cannula of the diameter of the aorta directly into the arch of the aorta and connect this by a wide rubber tube with the arterial resistance, replacing all the tubes throughout the system by tubes of the same diameter. Under these circumstances the heart was working at what appeared to be its maximum capacity and maintained a pressure at the beginning of the arterial system of 100 mm. Hg. without any resistance in the system at all, other than that offered by the flow of blood through the wide tubes.

Anatomically the vascular system may be compared, so far as its total cross section is concerned, to a double cone widening continuously from the aorta to the capillaries and narrowing again to the venæ cavæ. The tubes representing the aorta and vena cava in any artificial schema should therefore be the narrowest parts of the circulation. Under conditions of rest, however, we should have to represent the general tonic constriction of the arterioles by a number of stopcocks placed on the arterial side of the capillaries, impeding the escape of blood from the arterial system and so maintaining a normal arterial pressure. Under conditions of stress, when there is a maximal inflow and output, in our schema most of these stopcocks would have to be open, and in the animal there must be dilatation of the arterioles over so considerable an area that it becomes difficult to speak of a 'peripheral' resistance, the resistance of the whole system being no greater than if it consisted of a single tube with a diameter throughout about equal to the aorta. The work of the heart under these conditions is thus spent on driving the viscous blood at a great velocity through a comparatively wide system of tubes.

Finally it must be remembered that in one respect it is impossible to reproduce the conditions in the intact animal on our heart-lung preparation. In the intact animal the heart and lungs are normally under a negative pressure which, amounting only to a few mm. Hg. under normal circumstances, may increase to as much as 30 mm. Hg. during forced inspiration. When the heart is passive, as during diastole, this negative pressure will act on its walls and on the intra-thoracic portion of the great veins. During inspiration the descent of the diaphragm causes a positive pressure in the abdomen, so that the venous blood as it passes from the abdomen to the thorax passes into a great trough of pressure. If the negative pressure in the thorax is 30 cm. of water and the blood enters the thorax at such a rate as to maintain the pressure in the great veins at zero, *i.e.* equal to atmospheric pressure, the distending force on the auriculo-ventricular walls may be taken at 30 cm. of water. In our heart-lung preparation we could therefore only imitate this condition by passing in blood at such a rate that it exercised a positive pressure of 30 cm. of water on the heart walls. In the intact animal this great distending force will be active only during inspiration itself and will be lessened during expiration, though not abolished. In our experiments it must be active throughout the whole experiment. The larger positive pressure which we have to use in our experiments extends also to the vessels as they pass through the lungs, so that the working of a heart-lung preparation at its maximum efficiency is more dangerous for the lung vessels than it would be in the intact animal, and if persisted in too long tends to give rise to pulmonary œdema. It is possible to enclose the heart in a glass vessel and to expose it to a negative pressure of any desired extent, but we cannot conveniently devise a similar method of relieving the internal pressure on the lung vessels. But it is evident that the venous pressures we are warranted in adopting in our experiments in testing the maximum functional efficiency of the heart, must be much higher than the positive pressures observed in the big veins of the intact animal. In the latter circumstances 5 cm. positive pressure in the big veins might be equivalent to a positive pressure of 35 cm. of water in the heart-lung preparation.

Methods. All the experiments which form the subject of this article were carried out on dogs varying from 4 to 6 kilos. in weight. The heart-lung preparation was made as already described, the main difference in the apparatus employed in our experiments was in the size of the tubes employed. The arterial cannula had a bore of 8 mm. and was connected with the artificial resistance by a rubber tube with an internal bore of 8 mm. diameter. From the venous side of the resistance a rubber tube of similar bore passed to a glass spiral immersed in warm water kept at a constant temperature by means of a gas burner. On the connecting tube was a T-piece which could be opened so as to allow the whole blood coming through the arteries to flow out into a graduated vessel (Fig. 1). The time of filling of the vessel was taken by means of a stop-watch, thus giving the output of the left ventricle. From the glass spiral a tube led the blood into an inverted

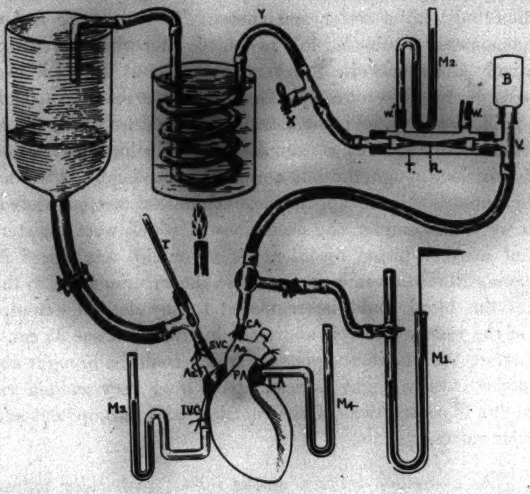


Fig. 1.

bell jar, from the bottom of which passed a rubber tube with an internal bore of 12 mm. to the venous cannula. The venous cannula in each case was chosen of the maximum size which could be inserted into the superior cava. When it was desired to determine the maximum output of the heart a still larger arterial cannula was used and was inserted directly into the arch of the aorta. The pressure in the inferior cava at its opening into the right auricle was measured by means of a long wide tube filled with hirudin solution, which was passed up from an opening in the inferior cava in the abdomen and tied in the inferior cava in the chest, so that its orifice lay close to the right auricle. This was connected with a water manometer. To the upper end of the water manometer a tube led to a piston recorder which served to show qualitatively differences in the venous pressure. In each case the piston recorder readings were controlled by a direct reading of the height of the fluid in the water manometer. The pressure in the left auricle was measured by inserting a cannula filled with hirudin solution into the left auricle through the left auricular appendage. It was secured by a ligature round the appendage. This also was connected with another water manometer, the excursions of which were registered by means of another piston recorder. A screw clip was placed on the tube passing from the venous reservoir to the superior cava, so that the inflow of the blood could be regulated to any desired extent. The height of the venous reservoir above the inferior cava was 35 cm. This was therefore the maximum pressure which could be brought about in the superior cava or right auricle, apart from very sudden rises of pressure due to contractions of the ventricle with incompetent auriculo-ventricular valves.

1. *The connection between venous inflow, ventricular output and venous pressure.*

The connection between these three quantities was investigated in various ways. In some cases the arterial pressure was kept absolutely constant by adjusting the air pressure round the arterial resistance so as to maintain the mean aortic pressure, as recorded on the kymograph, level. In most cases, however, the artificial resistance was maintained at a constant height, as judged by the height of the mercurial manometer connected with the air space outside the thin rubber tube which formed the resistance. Under these conditions, increased inflow and output of the heart caused an increased pressure in the aorta, since more fluid had to be passed in a given time through the artificial resistance. The rise of pressure was as a rule less than 30 mm. Hg., and in view of the constancy of the output of the left ventricle under varying arterial resistance, which has been shown in the paper by Markwalder and Starling, it seemed hardly necessary to trouble about an absolute adjustment of the mean aortic pressure.

The effect of altering the inflow was observed in various ways. In the earlier experiments the inflow was modified by altering the height of the venous reservoir. In the later experiments with larger tubes, the inflow was raised in steps from a minimal to a maximal amount by unscrewing the clip on the tube passing from the venous reservoir to the vena cava. So long as the heart continues to beat and maintain a circulation, its output must be equal to the venous inflow, and may be used therefore as a measurement of the latter. It is evident that the heart could not continue to throw out more blood than it received, and if it threw out less blood, each beat would leave the heart fuller than before, so that in a very short time the heart would be over distended and the circulation would come to an end. The results of four such experiments are given in the following tables.

Table I.

Exp. 1. Heart 84 gms. Small cannulæ in innominate artery and inferior vena cava.

Arterial B.P.	Pulse rate per min.	Height of venous reservoir (cms.)	Output per minute (c.c.)	Pressure I. V. C.
112	162	5	300	56
116	162	8	420	55
124	162	18	483	57
128	162	24	600	58
138	162	35	714	64
146	162	42	732	68
140	156	33	618	60
126	156	24	498	55
116	150	14·5	428	51
110	150	11	318	48
110	150	8	246	47
20	156	5	216	32
36	156	17	445	46
60	156	27	600	50
80	156	36	696	54
50	156	23	558	45
36	156	13	375	38
24	150	7	234	30
164	168	6	130	60
170	168	13	240	70
174	168	22	333	105
184	168	28	374	125
184	168	33	428	150
182	168	24	384	115
178	168	20	352	92
172	168	10	196	66

Exp. 2. Dog 6·45 kilos. Heart 44 gms. Cannulæ in aorta and inferior vena cava; cardiometer.

Temperature	Arterial B.P.	Pulse rate per min.	Pressure I. V. C.	Output per minute (c.c.)	Output per beat (c.c.)	Venous supply
36°	80	144	12	215	1·49	small
	92	144	32	850	6·00	+
	112	144	80	2000	13·90	+
	110	125	34	1620	12·00	–
	90	125	5	800	5·90	–
34°	80	120	0	105	0·87	small
	98	129	36	1070	8·30	+
	112	138	76	1580	11·40	+
35·3°	112	144	130	2000	13·90	+
	112	144	86	1620	11·20	–
	98	144	30	1000	6·95	–
36°	84	150	12	260	1·70	–

Exp. 3. Dog 5·15 kilos. Heart 56 gms. Cannulæ in innominate and inferior vena cava.

Temperature	Arterial B.P.	Pulse rate per min.	Pressure I. V. C.	Output per minute (c.c.)	Output per beat (c.c.)	Venous supply
34·5°	100	126	20	560	4·2	small
	110	126	60	1100	8·7	+
	110	129	100	1500	11·6	+
	96	132	150	1720	13·0	+
	90	132	230	2400	18·2	+
	90	135	250	3000	22·3	full

Exp. 4. Dog 5·75 kilos. Heart 52 gms. Cannulæ in aorta and inferior vena cava. Cardiometer.

Temperature	Arterial B.P.	Pulse rate per min.	Pressure I. V. C.	Output per minute (c.c.)	Output per beat (c.c.)	Venous supply
30°	72	92	12	270	2·72	small
	80	92	46	732	7·40	+
29·5°	84	92	74	1400	14·10	+
30°	90	114	94	1710	15·00	+
30·5°	86	114	46	1316	11·70	–
36·3°	70	162	20	666	4·10	small
36·2°	84	162	58	1316	8·40	+
	88	162	56	1670	10·30	+
35·5°	60	150	0	150	1·00	–

In the first of these the venous inflow was increased by raising the venous reservoir, in all the others by alteration of the clip on the wide tube used to connect the reservoir with a wide cannula in the superior vena cava, the level of the blood in the venous reservoir being about 35 cms. above the heart. These experiments show clearly that the output of any heart can be varied within wide limits by alteration of the venous inflow, and that within these limits it varies directly as the venous inflow. So long as the functional condition of the heart remains constant, the amount put out at each beat depends directly on the diastolic filling. If we are to understand the factors responsible for the power of the heart to adapt its output to the inflow, it is necessary to study the relation of the pressures on the venous side of the heart to the volume of inflow. This relation is shown in the curves (Fig. 2) in which the venous pressures in nine different experiments, as measured on the right side of the heart, are plotted against the output. It will be seen that as the inflow and output gradually increase, there is a slight rise of venous pressure with each increase. The height of the venous pressure does not, however, rise in a straight line but in a curve, becoming more rapid as the limits of the functional capacity of the heart are reached, until in some cases the heart becomes over distended, is unable to deal with the blood filling its cavities, and the output diminishes, although the venous pressure rises to a maximum. During the first part of the curve, with small to moderate output, we may say that the venous pressure, as measured at the entry of the vena cava into the right auricle, is mainly important in providing a quick flow of blood into the right auricle and

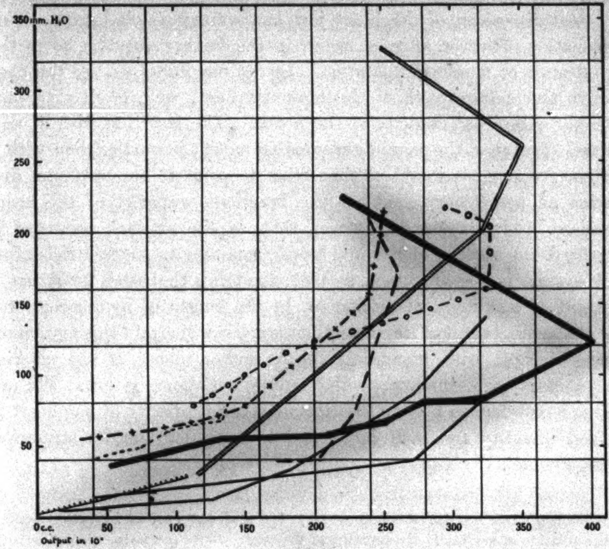


Fig. 2.

ventricle, so as to maintain a slight distending force on the relaxing ventricle, so that the blood is always present to follow the ventricular wall as it relaxes. As the inflow increases still further, the pressure begins to rise more rapidly and now becomes an effective factor in distending the ventricular wall and quickening the rate at which lengthening occurs after the end of systole. It is evident that there must be some limit to the process by which rise of venous pressure causes increased filling and increased output of the heart. This limit is probably reached when the venous pressure is high enough to produce maximal distension of the heart just as the next systole begins. This explanation, however, at once brings in the further question as to the significance of maximal dilatation. Is the maximum set by the connective tissue framework of the muscular heart wall, or does it vary with the functional capacity of the heart? The fact that with a high arterial resistance the maximum possible output is smaller than with a moderate resistance would at first seem to point to the optimum distension as being dependent on the functional capacity of the heart muscle. We have found, however, that, as the arterial resistance is increased, the amount of residual blood remaining in the ventricle after each systole is also increased, so that, assuming that each heart has a constant of maximum distension set by the length of its muscle fibres and the arrangement of its connective tissue, it will attain this maximum during diastole with increasing venous inflow sooner, if the arterial resistance is high, than when the arterial resistance is low. We are inclined therefore to believe that the maximum capacity of the heart is a fixed quantity for each heart and is dependent on its structural arrangements[1].

---

[1] The conditions urged in the footnote on p. 358 must also have an important bearing on the question of maximum distensibility of the heart. With increasing distension both cavities of the heart tend to become more spherical, so that whatever be the relations between tension of muscle fibre and intraventricular pressure in the contracted heart, they must tend to approach more nearly those ruling in a sphere the greater the distension of the heart. In the distended heart the tension on each muscle fibre will rise much more rapidly than the intracardiac pressure, and the more distended the heart, the greater will be the mechanical disadvantage under which the muscle fibres act, and this ever-increasing disadvantage must finally result in a condition in which the muscle fibres are unable to contract at all or to raise the intracardiac pressure higher than that existing at the end of diastole. The heart's action must then come to an end. It seems probable that the connective tissue framework of the heart would be so arranged as to limit as far as possible the attainment of this condition. Under normal circumstances it will be prevented by the restraining influence of the fibrous tissue of the pericardium, as was shown by **Barnard**.

Light is also thrown on the significance of the venous pressure in relation to the output of the heart by the experiments in which the rate of the heart beat was modified by alteration in temperature. In Exp. 4, Table I, the venous pressures were measured with varying inflow, first at about 30° C., and secondly at about 36° C. It will be observed that in the first series at 30° C. the venous pressures are uniformly higher than in the second series at a higher temperature with corresponding outflow. The results of an instructive experiment are also shown in Fig. 2 of Markwalder and Starling's paper. In this experiment the output was maintained constant, while the heart rate was altered by alteration in the temperature. With a moderate inflow the output remained constant between 72 and 156 beats per minute. The venous pressure however rose considerably with diminution in rate, namely from 38 to 110 mm. H_2O. In the second half of the same experiment, with an inflow of about 1250 c.c. of blood, the output also remained constant between 102 and 156 beats per minute. On further slowing the rate of the heart, the total output fell slightly to 1030 c.c. per minute. The rise of venous pressure was still more marked with the higher output. Starting at the highest rate of 156 at about 76 mm. H_2O, it rose to 190 mm. H_2O with a heart rate of 60. It must be remembered that with a constant output per minute, slowing of the heart rate means a proportional increase in the output per beat. In the experiment just quoted, the output per beat varied very considerably. This signifies that at the end of each diastole, the heart must be more distended when beating slowly than when beating more rapidly. To effect this distension a greater pressure is necessary. Since the output remained constant, one cannot speak here of a back pressure, the heart throwing out at each beat as much blood as it received between the beats. The sole significance of the rise of the venous pressure in this case is therefore to produce an increasing distension of the ventricle. It is evident, however, that in this way the heart will attain its maximum distension at a slow rate of beat with a smaller total inflow (*i.e.* inflow per minute) than at a more rapid rate of beat, so that there will be a limit to the degree to which a rise of venous pressure can be advantageous in causing an increased output of the heart. Prolongation of diastole, while not altering the total output of the heart with a moderate inflow, will tend to lower the maximum output per minute of the ventricles. With a constant inflow, increased rate of the heart will not alter the output of the heart. If, however, the venous pressure be maintained constant, increased rate of heart beat will cause increased output, since, with increasing rate the venous pressure can be maintained constant only by increasing the inflow. It would seem to follow from this argument that the shorter the diastole, and therefore the more rapid the contraction of the heart, the greater will be the maximum output of the ventricles. This is true also in the sense that a higher venous pressure is more effective in increasing the output at a high rate than at a low rate of beat. With shortening of the diastole the venous pressure becomes important in hurrying up the process of lengthening of the muscle fibres, which follows on the completion of systole. The phenomenon of the refractory period shows that the functional state of the cardiac muscle is not a constant quantity during the diastolic period. It is true that the contractile state passes off rapidly, but the disappearance is not an instantaneous one, so that the lengthening of the muscle under any tension would occupy a certain amount of time, which will be shorter the greater the tension. The shorter the diastole therefore, the greater the pressure which will be necessary within the heart cavities in order to obtain a maximum distension of these cavities before the onset of the increased systole. Thus in Exp. 3, with the heart beating rapidly, the output increased steadily as the inflow was increased up to its full amount. At the maximum output the venous pressure was 250 mm. H_2O. At this point this small heart was putting out 3 litres of blood per minute. It is evident that this increase of maximal capacity with increasing rate must have a limit, but the limit is rather physiological than mechanical and is set by processes of fatigue in the heart muscle. Evans has shown the large increase in metabolism of the heart with the increase of rate which attends rise of temperature, and also that the metabolism is increased by raising the mechanical demands on the heart muscle either by increased inflow or increased arterial resistance. As soon as the processes of recovery fail to keep pace with those of activity and the heart commences to show fatigue, its contraction will be less effective and there will be a larger amount of residual blood at the end of each systole. The heart will thus gradually increase in size until it is almost at its maximum distension at the end of systole, and a very small inflow of blood is sufficient to produce a maximal distension. Here then, nothing will be gained by increasing the venous pressure. The only way to save such a heart is to diminish the inflow and allow the heart time to empty itself and time to recuperate after its exertions.

The effect of moderate fatigue is well shown in Exp. 2. In this animal, with a heart of 44 gms., an output of 2000 c.c. per minute was obtained at the beginning of the experiment with a venous pressure of 80 mm. H_2O against an arterial pressure of 112 mm. Hg. About half an hour later in the same experiment, with the same rate of beat and the same arterial pressure, the same output of 2000 c.c. per minute was only obtained with a venous pressure of 130 mm. H_2O. This experiment shows us what fatigue of the heart means. In the fatigued heart a greater diastolic distension is necessary to produce a given output of blood at each systole than is required in the fresh heart beating at the same rate. This distension can only be produced by increasing the venous pressure. We shall have to return to this point when analysing the changes in the heart at each contraction which are responsible for the adaptive powers of this organ.

2. *The effect on venous pressure and ventricular output of exposing the heart to a negative pressure.*

As might be expected the positive pressures in the big veins near the heart may be kept much lower with a given inflow by exposing the heart to a negative pressure. For this purpose a glass cardiometer was introduced into the pericardium, which was tied tightly round it near the base of the heart. The side tube of the cardiometer was then connected through a mercury or water valve with a filter pump, so that a constant negative pressure in the vessel round the heart of about 10 cms. of water was maintained but which could be varied at will. The effect was to reduce the readings in the venous manometer whether connected with the right or the left side of the heart approximately by the amount of the negative pressure in the vessel surrounding the heart. The results of a few such experiments are given in Table II below, and demonstrate the truth of the statement made previously that a positive pressure of 150 mm. of water in the big veins, under the ordinary conditions of our experiment, is equivalent to a pressure of plus 50 mm. in the big veins and a negative pressure in the thorax of 100 mm., or to a positive pressure of zero in the big veins and a negative pressure in the thorax of 150 mm. of water in the animal with the intact thorax.

3. *The effect of varying the inflow on the pressure in the left auricle.*

In the preceding pages we have spoken of venous pressure and output from the arterial side, as if the heart consisted of only one auricle and ventricle. As a matter of fact in nearly all the protocols cited, the venous pressures were measured in the inferior vena cava, *i.e.* on the

TABLE II. *Effect of negative pressure.*

No. of exp.	Heart weight	Negative pressure	Arterial B.P.	Pressure I. V. C.	Left auricle	Pulse rate per min.	Output per min. (c.c.)
1	30·5	0	90	28—40	—	168	810
		6 mm. Hg	90	-80—-64	—	168	810
2	68·5	10 cm. H_2O	92	0—20	20—40	144	720
		0	92	40—50	64—80	144	720
		10 cm. H_2O	92	0—20	10—30	144	720
		0	96	200	200	144	600
		10 cm. H_2O	96	160	100	144	600
3	53·5	6 cm. H_2O	164	140	125	216	240
		0	164	170	200	216	240
4	49·5	14 mm. Hg	100	-35—-5	—	174	672
		0	100	40—80	—	174	672
5	106	0	100	20—30	50—65	102	860
		8 cm. H_2O	100	-20—-15	20—35	102	860

right side of the heart, while the output was measured from the left ventricle. It is evident that the filling of the right ventricle is alone directly influenced by the diastolic pressure in the right auricle and inferior vena cava, while the filling of the left ventricle must be a function of the diastolic pressure in the left auricle. It is therefore important to find out how far the pressure in the left auricle undergoes changes with altering venous inflow similar to those which we have found to occur on the right side of the heart. We give below records of five experiments, in which the pressures were measured simultaneously in the inferior vena cava and in the left auricle under varying conditions of inflow and systemic arterial resistance.

These results show that, in a heart that is in good condition, as the inflow is increased from a minimal to a moderate amount, the pressure in the left auricle rises at approximately the same rate as the pressure on the right side of the heart. This is what we should expect, since increased pressure in the inferior vena cava causes increased filling of the right heart and therefore increased output. The whole of this output is driven into the left auricle, so here again there must be a direct connection between venous pressure, diastolic filling and output. When, however, the demands on the functional capacity of the heart are increased by increasing the inflow into the right side of the heart to a maximum, marked differences may be found between the pressures in the right and left auricles, and these differences are especially pronounced when the heart fails or is on the point of failing. They are due to the fact that failure may commence on one side of the heart before the

TABLE III. *Left auricle.*

Exp. 1. Heart weight 70 gms. Cannulæ in inferior vena cava and left auricle.

Height of venous reservoir (cms.)	Blood pressure	Pulse rate	Output per minute (c.c.)	Pressure in left auricle (mm. H_2O)	Pressure in I. V. C. (mm. H_2O)
11·0	110	135	246	30	40
20·5	118	135	455	34	48
35·5	132	138	576	52	60
45·5	138	138	800	54	68
55·5	152	138	845	108	90
60·5	154	135	900	135	98

Exp. 2. Dog 4·15 kilos. Heart 34 gms.; cannulæ in inferior vena cava and left auricle.

Venous supply					
small	86	174	150	−20	20
+	88	174	430	−15	60
+	90	180	668	10	90
+	96	180	965	56	140
+	100	174	1178	79	160
+	105	177	1400	110	102

Exp. 3. Dog 5·5 kilos. Heart 47·5 gms.; cannulæ in inferior vena cava and left auricle.

moderate	86	186	690	40	30
+	92	186	2000	240	200
full	90	180	2100	400	260
−	90	180	1500	600	330

Exp. 4. Dog 7·2 kilos. Heart 60 gms.; cannulæ in inferior vena cava and left auricle.

moderate	94	200	684	2	78
+	90	200	1075	20	106
+	92	200	1980	55	152
full	92	200	2000	54	200
−	92	200	1760	55	210
−	90	192	780	0	64
+	90	192	1500	30	164
full	92	192	1880	54	204

Exp. 5. Dog 5·15 kilos. Heart 44·5 gms.; cannulæ in innominate artery, inferior vena cava, and left auricle; cardiometer.

small	92	192	312	0	54
+	92	186	600	12	67
+	94	186	912	38	84
+	96	186	1250	54	100
+	64	186	2400	80	120
+	66	180	2580	90	132
full	68	174	2460	108	132
−	88	156	6684	36	68

other, and that as a rule the side on which failure commences will be determined by the stress which is laid on it. Thus in the first experiment, with the not excessive output of 900 c.c. per minute for a heart of 70 gms., the pressure in the inferior vena cava was 98 mm. H_2O, while that in the left auricle rose to 135 mm. H_2O. In this case the arterial pressure, against which the left ventricle had to contract and drive out its blood, was 154 mm. Hg.—a high figure for a dog. The residual blood at the end of each systole in this case would be considerable, and the right heart had therefore to get up a pressure on the left side of the heart which would produce sufficient distension over and above the blood remaining in the left ventricle at the end of each systole.

In the third experiment, when the clip on the venous supply was opened to its maximum, the small heart of 47 gms. was evidently unable to deal effectively with the great volume of blood supplied to it. The failure here, however, affected the left side before the right; while the left side was passing out 200 c.c. of blood per minute, the pressure in the inferior vena cava was 260 mm. H_2O, while that in the left auricle was 400 mm. H_2O, *i.e.* higher than the height of the venous reservoir. In this case the pressure in the left auricle was directly due to the strongly contracting right ventricle, the left auricle being so to speak between two fires, the inadequately contracting left ventricle in front, and the strongly contracting right ventricle behind. When shortly afterwards the heart began to fail still more, so that it only sent out 1500 c.c. in the minute and the pressure on the right side rose to 330 mm. H_2O, the pressure in the left auricle rose to 600 mm. H_2O. On the other hand, conditions may arise which put a special strain on the right side of the heart. When the heart in the heart-lung preparation is made to work at its maximum efficiency, there is always a tendency to the occurrence of pulmonary œdema, and in such a case a resistance is offered to the flow of blood through the lungs and so to the contraction of the right side of the heart. In Exp. 4 it is evident that the right side of the heart was not so effective in sending on the blood as the left side was, so that the pressures in the left auricle throughout are much smaller than those in the inferior vena cava. The heart of 60 gms. could only put out 2000 c.c. of blood per minute with a maximal inflow, although the pressure in the right auricle rose to 200 mm. H_2O. The low figure of 54 mm. in the left auricle shows that the left ventricle was able easily to deal with the blood which the right ventricle could send on to it.

The last experiment quoted (Exp. 5) may be regarded as representing a heart in good condition. It will be noted that here the venous pressures[1] on the two sides rise proportionately with increasing inflow and diminish again proportionally as the inflow is diminished by screwing up the clip on the venous supply tube. It is therefore especially valuable in showing that the significance of rise of venous pressure in producing filling of the heart during diastole, which we have already discussed, applies to both sides of the heart. In a healthy heart which is contracting efficiently, a rise of pressure in the inferior vena cava may be interpreted as indicating a corresponding rise of pressure on the left side of the heart.

4. *The maximum functional capacity of the heart.*

As mentioned at the beginning of this paper, considerable doubt has existed, and still exists, as to the capacity of the heart to vary its output to an extent sufficient to provide for the respiratory needs of an animal during severe muscular exercise, so that recourse has been had to the theory, first put forward by Bohr and Henriques, that a considerable proportion of the oxidative processes of the body may occur in the blood as it passes through the lungs. Experiments by Evans and one of us have shown that there is no foundation for the ascription of such a function to the lungs, and that even the blood from an asphyxiated animal in passing through the lungs takes up only enough oxygen to saturate its hæmoglobin. In a paper published since the appearance of ours on this subject, Henriques, as a result of independent experiments, has come to the same conclusion. The conclusion necessarily follows that in severe muscular work the amount of blood put out by the heart and passing through the lungs must be increased five or six fold to carry the increased supply of oxygen which is taken in through the lungs under these conditions. The heart-lung preparation will obviously give us no information as to the amount of blood which can be regarded as normal for that heart in the animal at rest. In fact, such a figure is very difficult to obtain directly. It is quite true that we can expose the heart in an anæsthetised animal and determine by means of a cardiometer the changes of volume at each beat. The conditions of the experiment are, however, so abnormal that it is difficult to say what condition of the normal unanæsthetised animal would correspond with and give the same results as our experiments. The results obtained from the heart-lung preparation may serve, however, as a criterion of the reliability of the results on the output of the heart obtained by indirect methods, such as those employed by **Krogh** in man. In the heart-lung preparation the minimum output may be reduced, maintaining a normal arterial pressure, until the blood put out is only just sufficient to supply the flow through the coronary vessels, so that we can learn nothing from our preparation as to the minimum output of the heart in its normal relationships. We can, however, learn what is the maximum performance of a heart of any given size. A large number of determinations of the maximum output have been made. In the following table are given the results of nine of these on different animals. In many cases the maximum output was determined only towards the end of the experiment

---

[1] In all readings of venous pressure, whether on the right or left side of the heart, too much stress must not be laid on the absolute value of the reading. The opening of the inferior cava cannula was at the heart end of the inferior vena cava and therefore at the bottom of the chest cavity with the dog lying on its back. The left auricle cannula was tied into the left auricular appendage, and its orifice was therefore from 3—5 cms. higher than the orifice of the inferior vena cava cannula. The line of zero pressure of the cannula in the inferior vena cava was determined by cutting away the right auricular appendage at the end of the experiment. The registered pressure in this manometer gave therefore a fair idea of the pressure ruling in the part of the heart's cavity near its upper surface. The zero line of the cannula in the left auricle was taken as equal to that in the inferior cava. There may, therefore, be an error of 1 or 2 centimetres in the zero reading. On the other hand the conditions of the experiment would tend to give a higher reading in the inferior vena cava, since the flow into this vessel from the venous reservoir was continuous, whereas the left side of the heart was fed intermittently by the contraction of the right heart, and the main flow into the left side would occur during diastole, when the relaxed ventricle offered no, or a trifling, resistance to the inflow of blood.

TABLE IV.

No. of exp.	Weight of dog (kilos)	Weight of heart (gms.)	Arterial B.P.	Pressure I. V. C.	Pressure left auricle	Output per min.	Pulse rate per min.	Output per beat
1	5·3	40	104	170	—	1570	156	10·05
2		82 after adr.	132	45	—	2000	210	9·50
3	6·3	49·5	110	45	—	1720	174	9·85 before adr.
		negative pressure on heart	120	−20	—	1880	227	8·45 after adr.
			140	20	—	2000	222	9·00 more adr.
4	7·2	60	92	156	55	2000	204	9·85
5	5·5	47·5	72	260	400	2100	180	11·90
6	5·75	52	90	66	—	1715	114	15·00
			88	21	—	1715	162	10·2
7	6·45	44	112	76	—	2000	144	13·90
			later 112	130	—	2000	144	13·90
8	5·0	42	—	122	—	2400	180	14·3
9	5·1	56	90	250	—	3000	135	22·3

when the heart was already beginning to suffer from fatigue. The best result we have obtained is that given in Exp. 9. Here, a heart of 56 gms., *i.e.* about one sixth the weight of a human heart, put out for a considerable time three litres of blood per minute against a mean arterial pressure of 90 mm. Hg. and with a pulse rate of 135 per minute and an output at each beat of 22·3 c.c.

If we may take the output of a heart as proportional to its weight, a human heart under the same conditions would put out about 18 litres per minute, which is almost the same as the maximum output obtained by Krogh in his experiments on an athletic man[1]. There seems, therefore, no reason to doubt the conclusions of Zuntz, Plesch, and Krogh, that the extra amount of oxygen taken in during exercise is carried to the tissues by means of a proportional increase in the output of blood through the heart and in the flow of blood through the lungs. The whole question is one of venous inflow. Given sufficient variations of inflow, the heart can vary its output both per minute and at each beat within very wide limits. The circulatory problem in muscular exercise is not therefore how the heart drives the blood round, but the mechanism by which the blood is brought up rapidly from the peripheral parts of the body and driven at a high rate and appreciable pressure into the big veins of the thorax. The blood which comes to the heart will, until the heart suffers from fatigue, be thrown out by this organ.

It may be interesting to compare with these results those of two experiments in which we attempted to obtain some measure of the maximum efficiency of the heart in the whole animal. In these animals, which were anæsthetised in one case by urethane and in the other case by destruction of the brain, the output of the heart was determined by enclosing it in the cardiometer and measuring the excursions of the piston recorder connected therewith. In order to increase the venous inflow, normal saline fluid or blood was allowed to flow into the femoral vein so as to produce a plethoric condition. It will be observed that the highest output per minute obtained in these experiments was 1620 c.c. Under these conditions it is impossible to provide a sufficient venous flow, such as we can supply in the heart-lung preparation, or as in the normal animal is effected by the contractions of the muscles of the abdomen and limbs and the respiratory movements of the thorax.

---

[1] On the basis of the performance of the heart of 42 gms. in Exp. 8 the corresponding output of a heart of 300 gms. would be 17·1 litres per minute.

TABLE V.

Weight of dog (kilos.)	Weight of heart (gms.)	Arterial B.P.	Pressure I. V. C.	Pressure left artery	Output per min.	Pulse rate per min.	Output per beat
1. *Urethane.*							
5·5	40	—	—	90	1620	220	7·40
Saline run into femoral vein. Abdomen pressed.		—	—	45	1500	213	7·05
2. *Decerebrate.*							
4·95	68·5	155	50	30	810	135	6·00
Blood infused into femoral vein.		—	75	210	1500	188	8·00
Salt solution in femoral vein after adrenalin.		144	85	290	1620	220	7·50

CONCLUSIONS.

1. The output of the heart is equal to and determined by the amount of blood flowing into the heart, and may be increased or diminished within very wide limits according to the inflow.

2. The maximum output of the heart may amount to as much as three litres per minute for a heart of 56 gms. The maximum performance of the heart in the heart-lung preparation may therefore correspond with the maximum output observed by Krogh in man during severe muscular work.

3. With a minimal inflow the venous pressure on the two sides of the heart may be zero, and with increasing inflow rises at first only slightly, so long as the amount of blood flowing in is not more than sufficient to exert a minimal distension on the relaxing ventricles.

4. With further increase in the inflow, the venous pressure may be positive throughout diastole, causing an active distension of the heart and an increase in the rate of filling.

5. The maximum venous pressure induced by rise of inflow which can evoke corresponding increase in filling and output of the ventricles may amount on the right side of the heart to about 200 to 250 mm. H_2O.

6. The greater the arterial resistance, the higher will be the venous pressure for any given inflow.

7. The greater the arterial resistance, the sooner will the inflow attain its optimum, and the output its maximum. The maximum output is therefore higher the lower the arterial resistance, provided that the arterial pressure is high enough to maintain an adequate coronary circulation.

8. If the inflow is maintained constant, alteration of rate of heart beat does not alter the output per minute. Under the same conditions,

increase of pulse rate lowers the venous pressure. If therefore the venous pressure is maintained constant, increased pulse rate increases output (because to maintain the venous pressure constant with rising pulse rate, the inflow must be continually increased).

9. The greater the pulse rate, the larger may be the venous inflow without raising venous pressure beyond the optimum. The *maximum* output therefore increases with increasing rate of heart beat.

10. The optimum venous pressure is the amount necessary to produce "maximal" dilatation of the heart during the period of diastole. The maximum size of the heart depends on its structural arrangements, and in the intact animal is determined by the pericardium. It probably represents the size beyond which the heart could not dilate without causing its muscle fibres to act at considerable mechanical disadvantage in raising intracardiac pressure.

11. With a constant inflow, fatigue of the heart is shown by a rise of venous pressure accompanied by increased diastolic filling and mean volume of the heart, the outflow remaining constant.

12. The above statements as to venous pressure apply to both sides of the heart. When failure occurs under a maximal load, either the right or the left side of the heart may fail before the other side.

REFERENCES.

(1) **Zuntz.** Ztsch. f. klin. Med. LXXIV. S. 347. 1912.
(2) **Krogh.** Skand. Arch. XXVII. S. 100. 1912.
(3) **Yandell Henderson.** Amer. Journ. Physiol. XXXI. p. 352. 1912–13.
(4) **Markwalder and Starling.** This Journal, XLVIII. p. 345. 1914.
(5) **Knowlton and Starling.** Ibid. XLIV. p. 206. 1912.

THE TIME RELATIONS OF ACID PRODUCTION IN MUSCLE DURING CONTRACTION.

By H. E. ROAF.

(From the Laboratory of Physiology, St Mary's Hospital Medical School.)

For many years it has been known that muscular contraction causes a production of acid. By means of litmus paper du Bois Reymond demonstrated that resting muscle is alkaline whilst fatigued muscle is acid (1). Dreser (4) improved the demonstration by injecting acid fuchsin under the skin of a frog and by stimulating some of the muscles after twenty-four hours. The result of this experiment was that the resting muscles were colourless but the stimulated muscles became pink owing to the acid causing a change in colour of the indicator.

I have recently tried a similar experiment at the Port Erin Biological Station, showing the probable production of acid in a single contraction. Various indicators were dissolved in sea water and small medusæ placed in the solutions. The most successful experiment was in the case of neutral red, in which the medusæ stain a faint pink. At each contraction of the muscular plate the colour became a deeper red and then faded again. This change in depth of colour was too marked to correspond to the concentration due to the diminution in area of the umbrella during contraction; therefore the cause was probably the production of acid.

The importance of acid production during muscular contraction was emphasised by the work of Fletcher and Hopkins on lactic acid (5). They found that normal resting muscle contained very little or no lactic acid but that muscle which had been caused to contract contained appreciable amounts. The maximum amount was 0·19 p.c. for contraction and 0·33 p.c. for chloroform rigor. They also showed that after the contraction was finished the lactic acid was removed by some process which required the presence of oxygen. Their observations show that lactic acid is associated with muscle contraction but they do not prove that the acid is causative. Later work by A. V. Hill has shown that the amount of lactic acid produced is proportional to the tension developed in the muscle (7).

In view of the importance of this subject the experiments dealt with in this paper were undertaken and the acid production of muscle has been studied by means of electrodes in the hope that the time relations would show whether the acid appears early or late in the process of contraction. The results indicate that the formation of acid probably precedes the shortening.

An outline of the method has been previously given (10). It consists in placing two dissimilar electrodes on electrically symmetrical portions of the muscle (Fig. 1). One of these electrodes is a calomel electrode, *C*, consisting of mercury and calomel in contact with a Ringer solution of the same osmotic concentration as frog's blood and placed in contact with the muscle by a wick soaked in Ringer solution. The other electrode, *M*, is one that reacts to changes in the hydroxyl ion concentration. As the product of the concentration of hydrogen and hydroxyl ions is constant, a change in hydroxyl ion concentration is accompanied by the reciprocal change in the hydrogen ion concentration: therefore measurements of the hydroxyl ion concentration can serve to show the changes in the hydrogen ion concentration.

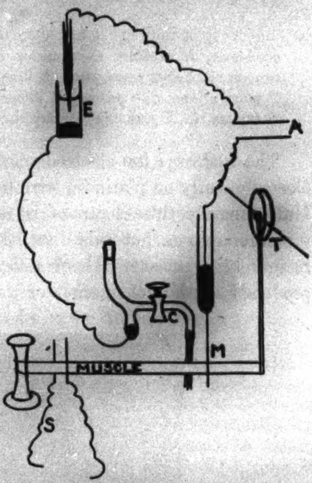


Fig. 1. Diagram representing the method of measuring the change in hydroxyl ion concentration of muscle. *S* stimulating electrodes, *T* tension lever, *C* calomel electrode, *M* manganese dioxide electrode, *E* capillary electrometer, *A* external compensating current.

The electrical potential produced by the contact of these two dissimilar electrodes with the muscle is balanced by an external current *A* and the muscle is caused to contract. As the electrodes are so arranged that two calomel electrodes on the same points at which the dissimilar electrodes are placed, showed no change of potential when the muscle contracts, any change in potential must be due to changes in concentration at the contacts of the electrodes with the muscle. The change of potential at the calomel electrode must be very slight as it is a contact between two liquids containing approximately the same concentrations of electrolytes and a slight change of concentration would have very little effect. Therefore any appreciable change of potential must be at the contact of the hydroxyl ion electrode and the muscle.

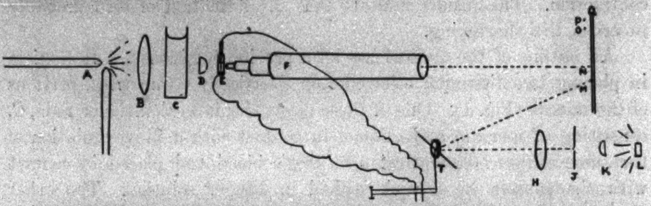


Fig. 2. Diagram showing arrangement of apparatus for recording electrical change and movement of muscle. *A* arc lamp, *B* condenser, *C* cooling trough, *D* bull's eye condenser, *E* capillary electrometer, *F* microscope, *N* level of mercury image on camera slit before contraction, *L* lime light, *K* condenser, *J* transverse slit, *H* lens, *T* tension lever with mirror, *M* image of slit *J* on mercury shadow on camera slit, *Q* camera slit, *O* signal in primary circuit of induction coil, *P* signal of time marker.

The hydroxyl ion electrode consists of manganese dioxide deposited electrolytically on platinum wire from a solution of a manganese salt (13). Unfortunately this electrode is said to be incorrect for quantitative measurements (12) but this does not interfere with its present use which is merely to measure the relation of the time at which the acid is produced to the contraction of the muscle.

Fig. 3. Manganese dioxide electrode positive, compensated. Increase in positive charge at manganese dioxide electrode beginning 0·013″ after beginning of the shortening of muscle. Time marker 0·01″.

The main difficulty is the preparation of the electrodes. The best method seemed to be to attach the electrode to the positive pole of a five volt current through a wire resistance of ten ohms, the resistance of the liquids being unknown. The negative pole was attached to another platinum electrode and the current passed for about one minute. After use they may be recoated by electrolysing again for about half a minute. After several recoatings it is better to clean them by soaking them in concentrated sulphuric acid and to start afresh. After the first coating of the electrode the electrometer shows a greater change of potential (Fig. 3) than after the subsequent coatings (Fig. 4). This agrees with the statement of Tower that a thin coating reaches equilibrium sooner than a thick one. In the present experiments the alteration of acidity is so rapid that equilibrium is not attained, therefore a thin coating shows a greater change of potential as the electrode approaches nearer to equilibrium in the same time.

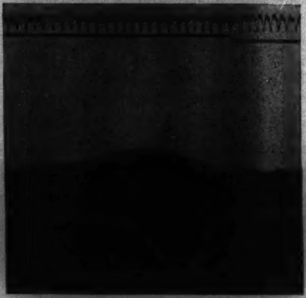


Fig. 4. Manganese dioxide electrode positive, compensated. Increase in positive charge at manganese dioxide electrode beginning 0·010″ after beginning of the shortening of the muscle. Time marker 0·01″.

The considerations which led to the selection of the manganese dioxide electrode are given below in order to indicate the points necessary for the selection of a better electrode.

1. The electrode must be independent of the pressure of oxygen as we do not yet know the variations in oxygen pressure during a muscle contraction. This excludes any form of oxygen or hydrogen electrode with either platinum or iridium. An oxygen electrode would show changes dependent on the use of oxygen by the muscle. A hydrogen electrode if saturated with hydrogen would remove oxygen from the tissues in contact with it so that the conditions of contraction would be abnormal.

Therefore an electrode of the second order containing an insoluble oxide is indicated. This is in equilibrium with the saturated solution of the corresponding hydroxide. The product of the concentration of the metallic and hydroxyl ions is constant and depends on the amount of the hydroxide in solution. Therefore a change in concentration of hydroxyl ions must be accompanied by the reciprocal change in the concentration of metallic ions and must cause a change of potential at the electrode. A decrease in hydroxyl ions will cause the electrode to become more positive.

2. The electrode must not contain anything which exercises a toxic effect on the tissue. Therefore electrodes such as those containing mercuric oxide are inadmissible (3).

3. The electrode must not form an insoluble salt with anything in the tissues. If it

does so the concentration of metallic ions will be so reduced that the product of the concentrations of metallic and hydroxyl ions will be less than that in equilibrium with a saturated solution of the hydroxide. That is the oxide would dissolve and the electrode become useless. Hence silver oxide which would form an insoluble chloride cannot be used.

4. The electrode must be so applied that it will not slip over the surface of the muscle as this would give rise to differences of potential. The movement of the muscle can be minimised by the use of an isometric or tension lever and the electrode should be applied in the form of a thin wire so that it will easily follow the slight movements of the muscle.

The electrical changes have been recorded by photographing the movements of the mercury column of a capillary electrometer (Fig. 2). The form of electrometer *E* was that designed by Dr Keith Lucas[1] for the stage of a microscope and the photographic plate was moved by a Lucas oil-piston (8). The movement of the muscle was recorded on the same plate by the projection of a beam of light from a mirror attached to the axis of the tension lever *T*. The mirror was placed in the same vertical plane as the capillary electrometer so that the spot of light was recorded at *M* on the shadow cast by the mercury column vertically under the record of the height of the mercury at *N*.

The tension lever consisted of a piece of piano wire clamped at the two ends, with a lever brazed on to it midway between the two clamps.

In front of the camera slit *Q* two signals were placed, so that their shadows were recorded on the portion of the plate unshaded by the mercury in the capillary. One of these *O* was placed in the primary circuit of the induction coil and its movement indicated the time at which the muscle was stimulated by a break induction shock. The second signal *P* was a time marker: in some experiments it was a magnetic signal placed in series with a tuning fork giving one hundred vibrations per second and in others it was a clock-work time marker recording fifths of a second.

The meaning of the electrical change.

Galeotti attempted to show an increase in acidity of the beating heart but he used hydrogen electrodes, hence as pointed out above, the tissues may not have been in a normal condition. His results were inconclusive as he found a negative, instead of a positive, charge during contraction. He found however that an indifferent electrode placed on the same portion of the heart gave a greater negative potential. The decrease in the negative potential, he ascribed to the increase in hydrogen ions at the hydrogen electrode (6).

---

[1] I wish to thank Dr Lucas for help and advice in fitting up the electrometer.

In the present experiments the electrodes were placed exactly opposite each other on a sartorius muscle. Preliminary experiments showed that indifferent electrodes in the same position gave no change of potential on stimulation (10). Further the curves show that the electrical change occurs at a different period of the contraction from the ordinary electrical change. A positive charge at the manganese dioxide electrode is equivalent to a negative charge at the calomel electrode. Since the record, as just mentioned, cannot be due to the ordinary negative change of muscle, a negative charge at the calomel electrode could be caused only by the presence of a negative ion with much greater rate of movement than the positive ions in the solution. The only negative ion that moves more rapidly than most positive ions is the hydroxyl ion and that would be much more effective in making the manganese dioxide electrode negative, hence an increase in hydroxyl ions cannot be the cause of the electrical charge. Therefore the positivity of the manganese dioxide electrode is not due to a negative charge at the calomel electrode.

As described above an increase in acidity should cause a positive charge at the manganese dioxide electrode. The manganese dioxide electrode was connected to the capillary and the movement of the mercury indicated that this electrode became more positive when the muscle contracted, thus indicating an increase in acidity; probably due to the liberation of lactic acid by the muscle during contraction.

The stage of the contraction at which the acid is produced.

The photographs show that the movement of the capillary begins soon after the beginning of the contraction. In those cases in which the movement of the mercury is not very great the mercury returns as the muscle relaxes, possibly indicating the removal of acid. When the movement of the mercury is very marked the mercury does not return so quickly. The difference in extent is probably due to the variability of the electrode (see p. 383) and it is probable that when the charge is great the delay in the return of the mercury is explained by the following considerations.

The acid diffuses from the muscle to the surface but the rate of contraction does not allow equilibrium to be attained. Thus it is possible that the amount of acid, in the muscle, may have reached its maximum and be decreasing but there may still be a higher concentration of acid in the muscle than on the surface, so that the acid may be still increasing at the electrode. In the same manner the electrode has not time to reach equilibrium so that the potential may still be increasing although the acidity at the electrode may have passed its maximum.

The latent period of the shortening of the muscle and the latent period of the movement of the mercury have been measured in a series of experiments and the results are given below.

TABLE I. *Difference in time, in seconds, between beginning of muscle shortening and of movement of mercury in the capillary.*

0·005
0·013
−0·003
−0·001
0·015
0·005
0·012
0·010
0·010

0·013 (Fig. 3)
0·018
0·010 (Fig. 4)
0·016
0·007
0·008
0·008
0·015

Average 0·0095 ± 0·0009

Although the movement of the capillary occurs after the commencement of the shortening of the muscle, the results are in favour of the acid development preceding the muscular contraction. The average delay was 0·0095 ± 0·0009 of a second, but we would, of course, be justified in taking the least difference if we could be certain that the individual observation were correct.

We must remember that there are several factors concerned in the delay of the electrometer record. The acid is produced in the muscle fibre. It must pass through the sarcolemma, and diffuse through any connective tissue between the muscle fibre and the electrode. After the acid reaches the electrode it must alter the equilibrium before the potential can be altered and finally the difference of potential must overcome the inertia of the mercury column.

The hypothesis relating the muscular contraction to acid development I have discussed elsewhere (11). I have assumed that the lactic acid combines with the protein of muscle to form an ionising salt. The protein ion with its positive charge cannot diffuse whilst the lactic ion with its negative charge can diffuse. Thus the ordinary electrical change in muscle would be produced. In this case the time of the ordinary electrical change indicates the time of the formation of the lactic acid salt of the protein. As the amount of any diffusion must be very slight the electrical charge is communicated to the electrodes immediately.

A salt of a weak base with a moderately strong acid is hydrolysed in solution with the formation of some free acid and a rise in the concentration of hydrogen ions. In the case of muscle the amount of lactic acid free to diffuse out is the small amount in equilibrium with the protein salt and not the total concentration of lactic acid.

The conditions of such "membrane equilibria" have been discussed by Donnan (2) but I cannot suggest any time interval for the process.

The length of time for the acid to diffuse from the muscle fibre to the electrode was estimated as follows. Sections of sartorius muscle were stained with van Gieson's stain and the thickness of the connective tissue measured by means of a micrometer eyepiece which had been standardised against a slide ruled in hundredths of a millimeter. On the superficial surface of the muscle there was a compact layer of connective tissue which was nowhere less than three micra in thickness. The deeper surface of the muscle had looser connective tissue and the muscle fibres beneath this connective tissue were not so evenly packed, hence the measurements were not so definite. A measurement of the compact layer of connective tissue on the superficial surface would be a fair estimate for the distance through which the acid must diffuse.

In the paper just referred to (11) the length of time for the diffusion of lactic acid was calculated to be 0·0004 of a second for a distance of one micron. The length of time is proportional to the square of the distance.

Therefore the time interval would be 0·008 of a second. This calculation assumes that the strength of acid is 0·01 normal and that sufficient acid diffuses to cause a change in colour of an indicator such as neutral red. In a membrane equilibrium the strength of acid would be much less than that actually found in the muscle by analysis and the time for diffusion should, therefore, be longer as the difference of concentration would be less.

We therefore see that most of the delay may be referred to the diffusion of acid from the nearest muscle fibre to the electrode.

The potential at the electrode is not an equilibrium condition such as that used in measuring ionic concentrations in the usual way, but it represents the changes at the electrode as it fails to follow the rapid changes in its surroundings. The hydrogen ion concentration is increasing as the acid diffuses from the muscle to the electrode and the potential is also increasing. The electrode would probably approach equilibrium logarithmically, and thus the commencement of the electrical change would begin very rapidly. The movement of the mercury in response to the difference of potential is also a logarithmic relation.

The following method partly suggested by Mr A. V. Hill was used to estimate the delay of the combined electrode and electrometer in recording the increase in acidity at the electrode. The wick of the calomel electrode was suspended transversely in front of the camera slit. On it was placed a piece of blotting paper moistened with Ringer solution and the manganese dioxide electrode was placed on top of the blotting paper exactly opposite to the slit. A tube containing 0·01 normal lactic acid was suspended above the electrode and the movement of the driving piston released a trigger so that the lactic acid was forced through a drawn out point in front of the camera slit.

The interval of time between the arrival of the shadow of the first drop of acid at the shadow of the electrode and the beginning of the movement of the mercury in the capillary electrometer was found to be 0·005 of a second[1]. If we add this time interval to the time calculated for the diffusion of acid from the muscle to the electrode (0·008″) we obtain 0·013 of a second which is more than the 0·0095 of a second found for the difference between the shortening of muscle and the movement of the mercury in the capillary.

Summation of contractions.

If the contraction of muscle is due to the production of acid and the acid is removed with the using up of oxygen we can suggest the following mechanism. Stimulation is followed by the production of acid, and as soon as the acid is produced oxidation commences. The contraction begins, but before complete contraction occurs the acid has so decreased that the relaxation commences. If by repeated stimulation the acid can be maintained, the process of contraction can proceed further, that is the height of contraction will be greater (9).

This state of affairs is shown by the electrode as there is a diffusion of acid to the electrode when the acid is in excess in the muscle and a diffusion of acid away from the electrode as the acid is removed by the muscle. As already mentioned it is only in the experiments in which the excursion of the mercury in the electrometer is slight that the inertia of the method allows the return to correspond in time with the muscle contraction.

With repeated contractions the variations of hydrogen ion concentration at the electrode will tend to disappear before the variations in acidity in the muscle. On p. 383 it was pointed out that the concentration of acid on the surface of muscle does not rise as high as in the muscle. Therefore, even if the acid in the muscle is reduced to the level of resting muscle, the diffusion of acid away from the electrode will be slower than its diffusion to the electrode as the difference in concentration will be less. A fresh development of acid in the muscle will therefore tend to cause a summation of acid at the electrode before complete summation of acid occurs in the muscle. Similar arguments show that the potential at the electrode will tend to fuse before the acid concentration at the electrode becomes uniform. The inertia of the electrometer will also tend to smooth away slight differences in potential especially if they follow each other rapidly.

---

[1] The addition of a drop of 0·01 normal lactic acid should give a shorter latent period than does the gradual increase in acid at the electrode as acid diffuses from the muscle to the electrode.

From these considerations we may expect to find that fusion of the potential due to acid will occur with a slower rate of stimulation than is necessary to produce complete tetanus, and this is in fact what the records show.

The muscle was stimulated by a series of stimuli caused by a vibrating spring in the primary circuit making and breaking contact with a mercury pool. The movement of the driving piston opened a short circuit in the secondary circuit. The reproductions show the contacts made by the vibrating spring and the contractions of the muscle indicate when the short circuit is opened. In a few experiments the time marking was in hundredths of a second, but in most it was in fifths.

All stages of summation, from a series of separate contractions with separate electrical changes to an almost complete tetanus with complete

Fig. 5. Manganese dioxide electrode positive, compensated. Two partially fused contractions with partially summed increases in positive charge at manganese dioxide electrode. Time marker 0·01″.

fusion of electrical changes, have been obtained. Figs. 5 and 6 illustrate two of these stages. The figures suggest that the acid production in muscle is associated with contraction, increasing during the mechanical shortening and decreasing during relaxation. With repeated stimuli the acidity at the surface of the muscle seems greater than with single stimuli and its summation occurs with a slower rate of stimulation than is necessary to cause complete tetanus.

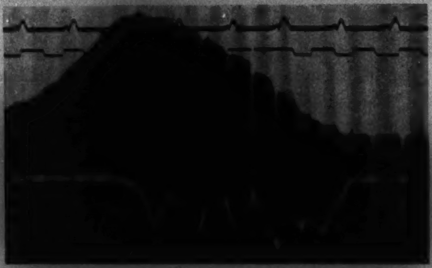


Fig. 6. Manganese dioxide electrode positive, compensated. Partial fusion of contractions with almost complete fusion of positive charges at manganese dioxide electrode. Time marker 0·2″.

SUMMARY.

1. An electro-chemical method is described for recording the increase in acidity when a muscle contracts.

2. The movement of the mercury in the capillary electrometer was found to follow the contraction by ·0095 of a second on an average. The time for the diffusion of acid is calculated to be ·008 sec. and that for the acid to cause a movement of the mercury in the capillary to be ·005 sec. These results appear to me to favour the view that the increase in acidity is the cause of the shortening of muscle.

3. Repeated stimuli lead to a fusion of electrometer curves due to increase in acidity before fusion of contractions occurs.

The expenses of this research have been defrayed from a grant from the Government Grant Committee of the Royal Society.

REFERENCES.

(1) **du Bois-Reymond.** Gesam. Abh. z. Algem. Muskel und Nerven Physik. Leipzig, 1877, pp. 5–36.

(2) **F. G. Donnan.** Ztsch. f. Electrochem. p. 572. 1911.

(3) **F. G. Donnan and A. J. Allmand.** Journ. Chem. Soc. Trans. XCIX. p. 845. 1911.

(4) **H. Dreser.** Cntrlb. f. Physiol. I. p. 195. 1887.

(5) **W. M. Fletcher and F. G. Hopkins.** This Journal, XXXV. p. 247. 1907.

(6) **G. Galeotti.** Ztsch. f. allg. Physiol. VI. p. 99. 1906.

(7) **A. V. Hill.** This Journal, XLII. p. 1. 1911.

(8) **K. Lucas.** Ibid. XXXIX. p. 214. 1909.

(9) **G. R. Mines.** Ibid. XLVI. p. 1. 1913.

(10) **H. E. Roaf.** Proc. Roy. Soc. B, LXXXVI. p. 215. 1913.

(11) **H. E. Roaf.** Ibid. (In the Press.)

(12) **W. A. Smith.** Ztsch. f. physikal. Chem. XXI. p. 93. 1896.

(13) **O. F. Tower.** Ibid. XVIII. p. 17. 1895.

ON THE ORIGIN OF THE "C" WAVE IN THE JUGULAR PULSE. BY JAMES M. McQUEEN AND A. W. FALCONER.

(From the Pathological Laboratory, Aberdeen University.)

ON the question of the origin of the *C* wave in the jugular pulse opposing views have been held. Mackenzie and Wenkebach hold that the *C* wave is due to the impact of the neighbouring carotid artery. The shock of the pulse in the artery is held to be transmitted to the cup receiver over the jugular bulb and to be impressed on the venous tracing as the *C* wave. Morrow, Fredericq, Bard, and Bachman believe that the *C* wave is truly auricular in origin and due to the sudden projection of the tricuspid valve into the cavity of the auricle during the presphygmic interval. This view is in agreement with the tracings of Porter of the auricular pressure where a first diastolic wave is clearly pictured as an auricular event, and its pressure value is stated to be 5 mm. of Hg. Ewing recently has shown that that *C* wave is not due to the impact of the aorta on the superior vena cava.

It is not disputed that in certain tracings an arterial pulsation can be impressed on the venous curve by altering the pressure of the receiving cup on the jugular vein. Lewis, after a careful recension of all the evidence, holds that "while it cannot be affirmed that the *C* wave is purely arterial or purely venous in origin, and while it cannot be denied that both factors may be contributory under certain conditions, it is highly probable that in one case the arterial and in another the venous element will predominate."

On the view that the *C* wave is due to the bulging of the tricuspid valve into the right auricle, it must be produced by the medium of the blood contained in the auricle; if the blood in the auricle is scanty, the wave will be small, and as it increases the wave will increase.

We accordingly determined to test the effect on the *C* wave of increasing the volume of blood passing to the auricle by the inferior vena cava.

This can be done easily by training the human subject to breathe deeply with the abdomen, and with as little expansion of the thorax as possible.

Fig. 1 shows that with the descent of the diaphragm—indicated as the rise in the tracing taken from the receiving bag tied on to the abdominal wall—several of the initial venous pulses show a marked increase in the size of the *C* wave.

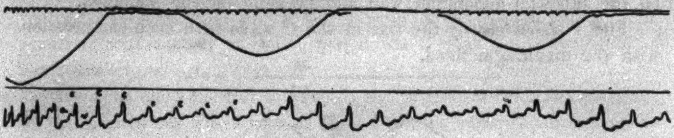


Fig. 1.

Fig. 2, giving simultaneous tracings of the radial pulse and of the jugular pulse, shows no increase in the size of the radial pulse wave synchronous with the increase in size of the *C* wave. A similar absence of effect occurs, when the carotid artery instead of the radial is taken, with the increase in size of the *C* wave in the jugular pulse. Consequently, we can conclude that the *C* wave is not enlarged because of an increased size of the carotid beat due to a more powerful ventricular systole. Hence the increase in size of the *C* wave in deep abdominal breathing is of auricular origin.

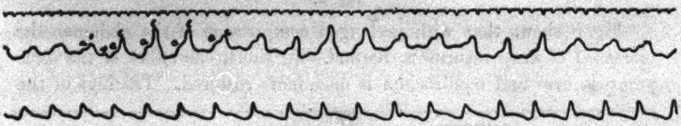


Fig. 2.

It will be noticed in Fig. 3 that the increase in size of the *C* wave disappears gradually before the abdominal wall recedes; that the *C* wave returns to normal while a deep abdominal inspiration is maintained. The descent of the diaphragm acts as a force pump pumping blood into

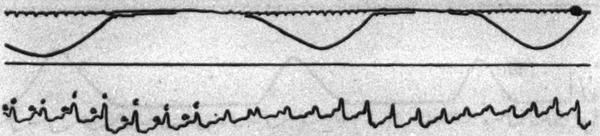


Fig. 3.

the auricle, but once the diaphragm is fully descended the stroke of the pump ends, the blood-flow into the auricle is no longer increased; and in consequence the *C* wave in the jugular pulse resumes its normal size.

We have further determined the effect of increasing the flow of blood from the inferior vena cava with the diaphragm fixed or in the act of ascent or descent. This can easily be done by placing the hand on the subject's abdominal wall and pressing on the abdomen.

Fig. 4 shows clearly the rise in the *C* wave with each compression with the diaphragm fixed.

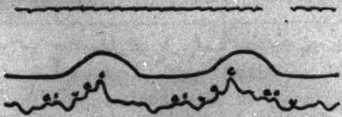


Fig. 4.

While Fig. 5 shows the same with normal breathing, the diaphragm being caught as it were in any position.

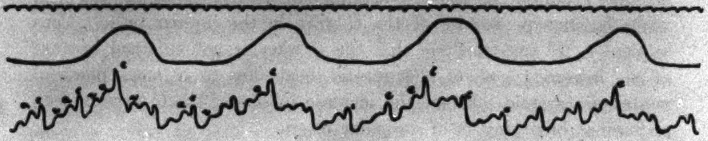


Fig. 5.

Fig. 6 shows that with prolonged compression of the abdomen the increased *C* wave returns to normal. In short, the effect of the force pump is over and equilibrium is once more restored. Tracings of the

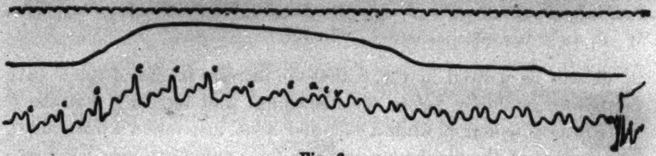


Fig. 6.

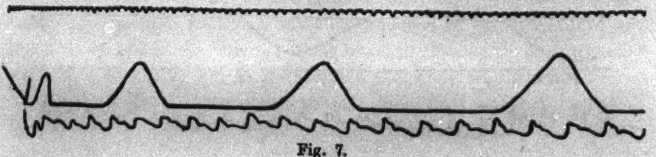


Fig. 7.

radial and of the carotid artery during the compression of the abdomen fail to show any marked increase in the force of the pulse wave. Cf. Fig. 7 for the carotid artery.

SUMMARY

An increase in the filling of the right auricle caused by an increase in the pressure and velocity of the inferior vena cava blood stream markedly increases the size of the *C* wave in the jugular pulse. The result is confirmatory of the view that the *C* wave is a phenomenon of auricular origin.

We conclude that the *C* wave in the jugular pulse is the resultant of the pressure of the stream of blood from the superior vena cava, the pressure of the stream of blood from the inferior vena cava below, and of the pressure due to the bellying out of the tricuspid valve with the rise of the ventricular pressure during ventricular systole.

REFERENCES.

Morrow. Arch. f. d. ges. Physiol. LXXIX. S. 442. 1900. Brit. Med. Journ. p. 1807. 1906.

Fredericq. Arch. Internat. Physiol. IV. p. 57. 1906–7. Ibid. V. p. 1. 1907.

Bard. Journ. d. physiol. et de pathol. gén. p. 454. 1906.

Bachman. Amer. Journ. Med. Sci. p. 674. 1908.

Porter. Journ. of Physiol. XIII. p. 513. 1893.

Ewing. Amer. Journ. of Physiol. XXXIII. p. 166.

Lewis. See "Further Advances in Physiology," edited by **Leonard Hill** (London), 1909, p. 101.

Delchef. Arch. Internat. d. Physiol. VII. p. 96. 1908.

Frey and Krehl. Arch. f. Anat. u. Physiol. S. 31. 1890.

MYOTHERMIC EXPERIMENTS IN SALT-SOLUTIONS IN RELATION TO THE VARIOUS STAGES OF A MUSCULAR CONTRACTION[1]. BY VIKTOR WEIZSÄCKER (*Heidelberg*).

(*From the Physiological Laboratory, Cambridge.*)

ALMOST the whole myothermic work of the schools of Heidenhain and Fick was devoted to the study of the relations existing between heat-production and mechanical energy under varying mechanical conditions, while the study of chemical and physico-chemical influences was neglected in a surprising degree. Many of the earlier investigators may have expected that an exact knowledge of the quantitative relations between (*a*) the liberation of heat and work, and (*b*) the mechanical conditions might lead to some simple law and thereby to a theory of contraction. Whatever the reasons for this view may have been, it cannot be denied that it led to a certain sterility. The subject of quantitative relations, although more advanced, is still the most difficult of all and has not yet justified the hopes based upon it. The problem has however assumed quite new aspects, since the discovery by A. V. Hill[2] of the facts of delayed heat-production. Hill examined the effect of oxygen and showed that the delayed heat-production is probably due exclusively to oxidation. The theory, that oxidation plays the part of a "recovery" process and takes place *after* the contraction, first brought forward by Hermann and based by him upon the facts of anoxybiosis, is thereby strongly confirmed. Myothermic experiment therefore has proved to be able to give some insight into problems lying out of the traditional path. Especially the comparison of gaseous exchange and myothermic experiment promises to become a source of information about certain questions regarding rather more the mechanism of muscular action, than its quantitative energetic properties. Questions of this description are the starting point of this investigation. The basis of any comparison of oxygen-consumption and heat-production would be according to this theory the assumption that oxidations follow the contraction and that the "delayed heat-production" is the outcome of oxidative, the "initial heat-production" more or less the outcome of non-oxidative processes.

---

[1] Preliminary communication: *Proc. Physiol. Soc.* p. xxxvi. 1914. (This *Journal*, XLVIII.)

[2] A. V. Hill. This *Journal*, XLVI. p. 28. 1913.

But one must remember, that as yet no direct proof exists, that the "initial heat-production" has not a different source in the presence and in the absence of oxygen, being an oxidation in the presence of O_2, and some other reaction in its absence, as in the case of certain facultative anaërobiotics. The mere facts of anoxybiotic work are no convincing proof against this suggestion. It also is not certain whether the initial heat-production in oxygen *includes* the outcome of oxidations in any degree. In this paper therefore I bring forward in the first place further evidence, that not only a part but probably the whole of the initial heat-production is due to a non-oxidative process.

The theory that oxidations play the rôle of a recovery process means, in terms of energetic consideration, that contraction in presence of oxygen involves a chain of at least two different transformations of energy[1], (1) energy derived from oxidations into an unknown energy x, and (2) energy x into mechanical energy. If this hypothesis be true, the task of further investigation of the heat-production of muscles will be, to differentiate between these two processes, to separate the heat-production of the first from that of the second transformation, to compare both and to investigate the different thermodynamic properties of these two systems. On the other hand, if we are able to show any difference between the behaviours (*a*) of oxidations, and (*b*) of initial heat-production, this will be evidence that our hypothesis of two systems holds. Now there is, *e.g.* for the heart-muscle, no difficulty in measuring the oxidations and the mechanical work, but no information is obtained in such experiments about the energy we called x. As I have shown in a previous paper[2] we also can influence the proportion between oxygen-consumption and mechanical energy in at least two different ways: (*a*) by cyanide this ratio is decreased, (*b*) by heating the muscle, by alcohols and hypotonic solutions, it is unaltered or even increased. The next question is: Are such changes of the efficiency due to changes of efficiency in the first, or in the second transformation? Are these influences working upon the oxidative recovery-processes or upon the contraction-processes *sensu strictiori*?

---

[1] Cf. A. V. Hill, *l. c.*

[2] Weizsäcker. Preliminary Communication: *Naturhist-med. Verein*, Heidelberg. *München Med. W.* No. 7. 1914.

Since, as I shall show below, the initial heat-production can now be regarded as the pure outcome of the latter transformation, we shall be able to try the above-mentioned effects of temperature, cyanide, etc. upon the initial heat-production and then compare our results with their effects upon oxygen-consumption. For the purposes of such investigation I have had to undertake the examination of muscle in solutions of different drugs.

Method[1].

It seems that the attempt has never yet been made to carry out myothermic experiments in salt solutions. For my purpose to investigate the effect of dissolved substances it was indispensable to keep the muscle in solutions. This step could scarcely have been made, if the sensitivity of modern methods had not been greatly improved by the use of Paschen's galvanometer (Bürker, A. V. Hill) and the invention of thermopiles with 2 × 30 and more junctions (A. V. Hill). The arrangement was the following (Fig. 1). A large cylindrical Dewar-flask of 40 cm. depth and 8 cm. width contained 500 c.c. of Ringer's fluid. The flask was covered by a thick wooden stopper. The heat-insulation was improved by the large column of air between the Ringer's fluid and the stopper. A strong glass-rod is fixed in the stopper and holds at its lower end the thermopile. The latter is of form similar to that described and pictured by Hill[2]. It has a slit (*s*) through which the muscle (double preparation of semimembranosus) is pulled and in which the bone-end is held fast. At *P*, *W* there are two platinum wires serving as stimulating electrodes. The wires of the thermopile and the stimulating wires pass through the stopper and are connected with the galvanometer and the induction-coil respectively. Thermopile and wires must be well insulated by shellac. I used the iron-shielded Paschen galvanometer, which has been described by Hill (*l.c.*). The glass-tube reaches the bottom of the flask and fulfils three purposes: (1) it serves for bubbling air or other gas through the fluid; (2) for removing the fluid (as a siphon) and replacing it by a fresh one; (3) for adding concentrated solutions of substances to be tried. The bubbling also is useful for stirring, but it can be replaced by a special glass-stirrer. Through another hole passes the thread from the muscle-ends to the recording lever and one more hole contains a thermometer (*Th*).

---

[1] Preliminary Communication: A. V. Hill and V. Weizsäcker. *Proc. Physiol. Soc.* p. xxv. 1914. (This *Journal*, XLVIII.)

[2] This *Journal*, XLVI. p. 42. 1913.

Sensitivity. The thermopile had 2 × 35 junctions of iron and constantan. For a given sensitivity of the galvanometer and a single twitch the deflections in Ringer's fluid are about 5 to 10 times smaller than in air. The sensitivity being very high there is no difficulty in obtaining deflections of sufficient size even for very small twitches. The reason why the readings are comparatively small in Ringer's fluid is that the heat-capacity of water is some 3000 times as great as that of air, and therefore the loss of heat after a twitch will be quicker. After having changed the Ringer's fluid, one usually has to compensate the galvanometer in order to get the light-spot on the scale again. It was advisable therefore to try whether the sensitivity of the whole apparatus is changed or not by the slight movement of the magnet. By measuring the deflection effected by removing and replacing a piece of iron into a precisely definite position near the galvanometer I found that there are no changes of sensitivity sufficient to change the results in any way.

Disturbances of the galvanometer. These movements have three causes: (1) shaking by the traffic; (2) disturbances of earth-magnetism and electro-magnetism; (3) movements of heat along the thermopile. (1) and (2) behave in this method in no peculiar way, and seldom caused any trouble. But factor (3) demands special attention. It has not been possible to avoid a very slow but quite regular wandering of the light-spot. It is easy to estimate the speed of this movement during 1 or 2 minutes before an observation is made, and to correct the readings. The correction was almost always less than 10 °/₀, and throughout many experiments less than 1 °/₀ of the whole reading. But I have not been able to detect the cause why this creeping of the galvanometer by movements of heat in the flask occurred in different degrees in different experiments. Immediately after change of solution these movements are very quick; but after 10 minutes they have decreased sufficiently for observations to begin, and after 20 or 30 minutes the maximum of quiescence is reached.

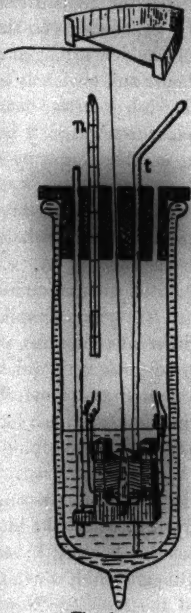


Fig. 1.

Different temperatures. I have used the Dewar-flask not only in order to avoid the moist chamber, but especially in order to work in temperatures above and below the room temperature. The investigation from 0° to 20° never fails, and the galvanometer was usually at 0° almost as still as at 15°. But from 18° upwards an increasing lack of quiescence is observed, so that results obtained above 25° are not reliable.

If the difficulties are kept in mind, which Fick had to undergo in his experiments on the effects of different temperatures, the advantages of the new arrangement are obvious. Fick's experiments lasted many hours and obviously all the muscles were affected by time or "fatigue." The use of Dewar-flasks permits one easily to compare four different temperatures within 2 hours, and one may proceed from one temperature to another simply by changing the Ringer's fluid brought to the required temperature.

The drawback of this method is, that it has not been possible to investigate the whole shape of the curve of heat-production, as has been done by A. V. Hill. It only allows an investigation of the "initial heat-production," *i.e.* the maximum deflection. Its advantages are (1) great simplicity and the possibility of investigating many different conditions, physical as well as chemical, and of changing these conditions very quickly; (2) the muscles being in Ringer's solution the products of metabolism are allowed to diffuse away. Therefore the muscle keeps fresh for a longer time, and this can be aided by (3) the possibility of keeping it at low temperatures.

Action of drugs. The heating of the thermopile depends in the first place on the superficial fibres of the muscle lying immediately upon it, whilst the inner and the opposite parts of the muscle take part in the heating in a smaller degree. This is the case especially in Ringer's fluid, which easily carries away the heat from the free surface of the muscle. Now if we put in, *e.g.*, a certain amount of alcohol, naturally the superficial fibres will be altered at first, whilst the inner parts are still normal. It might occur that the heat-effect (dependent on surface fibres) would be already strongly diminished, at a time when the total tension effect is not yet noticeably altered. This cause of error will probably be counteracted by the greater difficulty with which the drugs can diffuse between the thermopile and the muscle lying upon it. It is not possible to state which effect preponderates. It is generally known that cyanide and alcohol penetrate the cells very quickly. This is different with water and salts; but in both cases I have always preferred very thin muscles. Nevertheless it will be advisable to keep these possible causes of error in mind when we analyse an experiment. No objection is to be made to observations, in which the heat and the tension developed keep constant during 10 or 20 minutes. This proves that equilibrium is reached; but in a muscle continually changing its properties, no far-reaching conclusion should be drawn from the proportion between heat and tension.

Mechanical records. Heat and tension. The free ends of the muscles were directly connected with the tension-lever described by Hill[1]. I always used isometric twitches. I do not wish here to enter into the question, if and how far the production of tension can be regarded as an indicator of the greatest work that could be done if the muscle were allowed to shorten. Even if the tension set up were more or less proportional to the greatest possible work under normal conditions it might be doubtful whether this holds for pathological conditions. For the present purpose it is sufficient that the proportion between heat and tension set up shows under different conditions very definite alterations. It is the *way* in which such alterations occur that gives us already almost as much information as a quantitative measurement of heat and maximal work would do, for even then no quantitative application of thermodynamics would be allowed. Tension of course represents in no way any form of energy, neither potential energy nor any other form; but we cannot doubt that *e.g.* a decrease of tension set up always shows a decrease of mechanical energy.

Since the muscle is not allowed to shorten, no work can be done, and the whole of the mechanical energy is finally transformed into heat. Therefore the heat H, we observe, is always the sum of mechanical energy plus the heat which is directly produced, in other terms, "H" is an indicator of the total energy liberated in the first stages of one complete contraction-process. The initial tension was low, 4·8 grms., and was kept constant throughout one experiment. Small changes of initial tension, due generally to changes of elasticity in the muscle, are evidently of no noticeable influence upon heat and tension set up.

The figures given below under the heading tension (T) represent mm. deflection of the Hill-lever. The calibration of the lever gave the following results (Table I):

---

[1] A. V. Hill. This *Journal*, XLVI. p. 452. 1913.

TABLE I.

Load in grms.	Deflection in mm.	Difference for 10 grms. in mm.
1	0·2	—
2	0·35	—
5	0·9	1·8
10	1·8	1·8
20	3·5	1·7
30	5·3	1·8
40	7·0	1·7
50	8·8	1·8
60	10·5	1·7
70	12·2	1·7
80	13·9	1·7
90	15·6	1·7
100	17·2	1·6

This table shows that 1 mm. deflection indicates about 5·7 grms. tension. Within the range of deflections appearing in this paper there is therefore only a very slight drop of proportionality between deflection and tension, from about 1·8 to 1·65 or 1·7 mm. deflection for 10 grms. tension, and correction has therefore been thought unnecessary.

PART I. EFFECT OF OXYGEN.

As A. V. Hill[1] points out, the true curve of the heat-production is altered by absence of oxygen in its later part only, very distinctly only from 20 or 30 seconds after the contraction. This suggests that the "initial heat-production," indicated by the maximum deflection (which is reached 5 to 7 sec. after contraction), is probably due to non-oxidative processes. However his method was necessarily a comparison of the *shapes*, not of the absolute values. And moreover no method exists of investigating the shape during the first few seconds. So there is no evidence that absolute height as well as shape during contraction and the first few seconds after it might not be more or less different in oxygen and in nitrogen. One can see, however, from some of Hill's experiments, that the maximum deflection has not been very different. But there were no mechanical records, and here it is desirable also to know whether the mechanical response also is the same in both cases. Therefore in my first experiments special attention was paid to the absolute amount of initial heat-production and the development of tension with and without oxygen.

Two methods have been used: (*a*) alternate observations were made in Ringer's fluid saturated (1) with oxygen, and (2) with nitrogen; (*b*) the oxidations were stopped by cyanide. The results are exactly the same in both cases. I always used maximal isometric twitches produced by single induction-shocks. Further details are given in the protocols. The heat (*H*) is given in mm. deflection (scale division). Although 1 c.c. of water saturated with air contains less than $\frac{1}{30}$ of the oxygen present in 1 c.c. of air, the oxygen-*pressure* inside the muscle-cell will naturally be the same whether we put the muscle in a certain gas (or mixture of gases) or in a Ringer's fluid in equilibrium with such gas.

---

[1] This *Journal*, XLVI. p. 28. 1913.

Some preliminary experiments showed that the initial heat-production is the same in ordinary Ringer's fluid and in oxygenated Ringer's fluid. Here I only give experiments showing the influence of pure oxygen and pure nitrogen.

Exp. 1. *Semimembranosus* (here as always double preparation), 220 mg. 15° C. inside Dewar-flask. The Ringer solution was kept in a wide flask with a narrow opening. The gas (O_2 or N_2) bubbled through for some time, after which the flask was shaken for several minutes. This was repeated two or three times. Then the solution was poured into the Dewar-flask and the air in the flask replaced by the same gas; gas was bubbled through the solution at the same time for several minutes. This was repeated during the periods (*a*), (*b*) etc. Each "observation" represents a single twitch.

Period	Observ.	Time	*H*	*T*	$\frac{H}{T}$
(*a*) in O_2—	1	12.6	36	8·2	4·4
begins at 11	2	12.11	37	8·8	4·2
first observ. 12.6	3	12.18	38	8·9	4·3
last observ. 12.50	4	12.26	39	9·6	4·1
	5	12.36	38	8·7	4·4
	6	12.39	39	9·0	4·3
	7	12.40	39	9·4	4·1
	8	12.50	38	9·0	4·2
(*b*) in N_2—	1	2.41	34	8·3	4·1
begins at 12.55	2	2.46	32	8·0	4·0
first observ. 2.41	3	2.58	35	8·8	4·0
last observ. 3.17	4	3.0	36	8·2	4·4
	5	3.9	35	8·0	4·4
	6	3.13	33	8·2	4·0
	7	3.17	35	8·3	4·2
(*c*) in O_2—	1	3.40	39	7·6	5·1
begins 3.25	2	3.47	35	7·5	4·7
first observ. 3.40	3	3.55	36	7·9	4·6
last observ. 4.25	4	4.5	34	7·4	4·6
	5	4.17	35	7·8	4·5
	6	4.25	35	7·8	4·5
(*d*) in N_2—	1	4.48	32	8·1	4·0
begins at 4.30	2	4.52	33	8·1	4·1
first observ. 4.48	3	4.56	31	8·1	3·8
last observ. 5.4	4	5.0	34	8·0	4·3
	5	5.4	34	8·0	4·3
(*e*) in O_2—	1	5.21	33	7·4	4·5
begins 5.10	2	5.28	34	7·7	4·4
first observ. 5.21	3	5.36	36	8·0	4·5
last observ. 6.8	4	5.43	37	8·1	4·6
	5	5.48	35	8·0	4·4
	6	5.54	35	8·0	4·4
	7	6.3	37	8·2	4·5
	8	6.8	36	7·7	4·7

This experiment shows (1) that for more than 6 hours there is no noticeable decrease either of initial heat-production or of tension set up. (2) That no noticeable difference can be stated between oxygen and nitrogen. Small divergences may appear, but the possible error being between 5 and 10 °/₀ we therefore can say, that within the limits of error the absence of oxygen does not influence the initial heat-production. (3) The proportion between heat and tension (H/T) is equally independent of absence of oxygen. Two objections can be made. There is no evidence as to when the diffusion of gases is completed throughout the muscle substance. But if any changes were going on we could not expect such constant figures. Furthermore, for instance, an experiment given by Hill[1] shows that the influence of oxygen upon the recovery heat-production is fully developed at the latest after half an hour in oxygen, perhaps earlier. In the next place no precaution has been taken to replace the last traces of oxygen in the N_2-periods. These objections will be refuted by the following experiments (Table III), in which the oxidation-ferment itself has been checked by cyanide. The cyanide enters the cells almost instantaneously and has the advantage over the usual anoxybiotic condition that its effect cannot be objected to by any assumption of intramuscular oxygen store. As I have shown[2] the oxidation of the heart-muscle of the frog is reduced to less than 10 °/₀ of the original value by 0·0005 N KCN. I used in consequence solutions of 0·001 N up to 0·003 N. No living cells are known, whose oxidations resist these concentrations.

Exp. 2. *Semimembranosus* conditions as above, oxygenated Ringer's solution. A stock solution of 0·77 N KCN in H_2O was kept and controlled by titration with silver nitrate.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) in O_2	1	4.25	32	9·7	3·3
	2	4.34	32	9·8	3·3
	3	4.43	29	9·4	3·1
	4	4.52	27	9·1	3·0
	5	5.5	29	9·0	3·2
(*b*) 0·00077 *n* KCN	1	5.20	25	8·8	2·8
	2	5.25	28	8·9	3·2
	3	5.32	27	8·8	3·1
	4	5.46	29	8·8	3·3
	5	5.51	29	9·1	3·2
(*c*) 0·0015 *n* KCN	1	5.59	28	8·8	3·2
	2	6.4	28	8·8	3·2
	3	6.8	29	8·9	3·3

[1] This *Journal*, XLVI. p. 65, Exp. 3. 1913.

[2] Weizsäcker. *Pflüger's Arch.* CXLVII. p. 135. 1912.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*d*) 0·003 *n* KCN	1	6.21	32	8·9	3·6
	2	6.26	28	8·2	3·4
	3	6.29	28	8·3	3·4
	4	6.37	28	8·2	3·4
(*e*) in O_2	1	6.51	27	7·9	3·4
KCN removed	2	6.55	28	7·9	3·5
O_2-Ringer sol. filled in	3	7.0	27	7·8	3·5
	4	7.3	28	7·8	3·6
	5	7.7	27	8·1	3·3
Exp. 3. *Semimembranosus*. As Exp. 2.					
(*a*) in O_2-Ringer	1	11.33	47	13·6	3·5
	2	11.36	47	13·6	3·5
	3	11.39	47	13·6	3·5
	4	11.42	48	14·2	3·4
	5	11.46	49	14·1	3·5
	6	11.49	43	12·0	3·6
	7	11.53	49	14·0	3·5
(*b*) 0·002 *n* KCN	1	12.5	46	12·6	3·7
	2	12.9	49	13·0	3·8
	3	12·12	49	13·0	3·8
	4	12.25	48	12·5	3·8
	5	12.39	49	12·8	3·8
	6	12.55	42	12·1	3·5
	7	12·59	42	12·1	3·5
	8	1.5	38	10·6	3·6
	9	1.10	36	10·5	3·4
	10	1.23	40	10·2	3·9
	11	1.30	34	9·9	3·4
(*c*) in O_2-Ringer	1	1.47	37	9·1	4·1
	2	1.50	38	9·1	4·2
	3	3.32	33	8·1	4·1
	4	3.34	32	8·0	4·0

In another experiment the figures for *H* and *T* were more irregular, because an imperfect make and break key (no platinum) was used. The ratio however of *H*/*T* was fairly constant.

In Exp. 3 the muscle was exposed for a long time to cyanide (1½ and 2¾ hours). In consequence, the contractility drops after about one hour, but the ratio between *H* and *T* remained constant, since the initial heat-production dropped in the same degree. The diminution of contractility was not reversible.

From these experiments we therefore can draw exactly the same three conclusions as before. The initial heat-production is not appreciably altered by cyanide neither is the mechanical response. Only when the anoxybiosis lasts a longer time, there is a decrease of heat and tension set up in the same degree.

It would be very curious, if a muscle produced exactly the same heat and the same mechanical energy, in one case by oxidations, in the other by some other reaction. Such a thing is not seen in any other cell, whose functions are more or less facultatively anaërobic. For further investigations we can now assume that the initial heat-production, measured by the maximum deflection of the galvanometer, is due, whatever the oxygen pressure may be, to a non-oxidative process, and that (*a*) the amount of initial heat, (*b*) the tension set up and therefore also (*c*) the proportion between them is independent of the oxidations going on in the muscle.

PART II. EFFECT OF TEMPERATURE.

In this part I proceed to study the thermodynamics of the transformation of the potential energy x into mechanical energy. The statement of Part I enables us, as I have said above, to investigate this transformation. Speaking of "heat" in this part I refer always to the "initial heat-production." Throughout I have used the isometric twitch.

Only two investigators have dealt with the influence of temperature upon heat-production. Fick[1] certainly had the very greatest difficulties in his experiments. We need not call attention to the weak points of his work because, although Fick himself brings forward a definite result, a more close examination of his own figures does not seem to support the conclusions he draws. Besides this only two experiments are reported *in extenso* and in the protocols we miss the important data of time. His conclusion is that at a higher temperature more heat is liberated by a maximum twitch, although the mechanical response does not increase. The difference of heat-production at different temperatures was however—as Fick states—much smaller in isometric than in isotonic twitches. Now isotonic experiments are always, as A. V. Hill points out, exposed to the objection of slipping over the junctions. If we only consider Fick's isometric experiments, we find (omitting his first experiment) the following average values of 7 experiments:

Cold muscle 49·3 scale—div. (temp. below 10°).

Warm muscle 43·6 scale—div. (temp. beyond 19°, in most cases beyond 27°).

The averages of Fick's results therefore lead to a different conclusion from that reached by Fick himself. But the single experiments give very variable results and obviously the whole give no definite result at all.

In the next place Hill[2] has given some experiments, which lead to a directly opposite result: for a given amount of tension he obtains more heat-production at a lower than at a higher temperature. Since

---

[1] Fick. *Ges. Schriften*, II. p. 329. Würzburg, 1903. (*Verh. d. phys.-med. Ges. Würzburg*, N.F., Bd. XXX. p. 61.)

[2] A. V. Hill. This *Journal*, XLII. p. 22. 1911.

Hill also worked in air his experiments lasted several hours and a proceeding influence of time or "fatigue" hindered a comparison under similar conditions. However it will be seen, that in spite of this the same result is obtained under the improved conditions of my own experiments. The latter allow us to examine two questions hitherto not kept apart, namely (1) Is the absolute amount of energy (heat and mechanical energy) liberated in a single twitch different at different

Exp. 1. *Semimembranosus*, single maximal twitches by break induction-shocks.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) 16° C.	1	4.19	35	8·8	4·0
	2	4.23	34	8·7	3·9
	3	4.27	33	8·6	3·8
	4	4.30	35	8·7	4·0
	5	4.34	35	8·7	4·0
(*b*) 22·5°	1	4.55	27	7·7	3·5
	2	4.56	27	7·8	3·5
	3	5.3	26	7·5	3·5
(*c*) 14·5°	1	5.36	40	7·3	5·5
	2	5.39	42	7·4	5·7
	3	5.44	43	7·5	5·7
(*d*) 25·5°	1	6.18	26	7·0	3·7
	2	6.21	28	6·8	4·1
	3	6.24	30	6·8	4·4
(*e*) 12°	1	6.41	41	8·0	5·1
	2	6.46	45	8·1	5·6
	3	6.50	47	8·1	5·8
	4	6.53	51	8·0	6·4
	5	6.56	49	8·1	6·0
	6	6.59	53	8·0	6·6
	7	7.2	53	8·0	6·6

Exp. 2. *Semimembranosus.* Initial tension 4·0 grms. wt.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) 15°	1	10.3	44	9·0	4·9
	2	10.7	44	9·6	4·6
	3	10.10	44	8·5	5·2
	4	11.14	49	9·0	5·5
	5	11.17	50	9·0	5·6
	6	11.20	49	9·5	5·2
	7	11.24	49	9·5	5·2
	8	11.31	47	9·5	5·0
(*b*) 10°	1	12.5	68	10·5	6·5
	2	12.12	70	10·5	6·7
	3	12.16	74	10·5	7·0
	4	12.21	74	10·3	7·2
	5	12.26	72	10·3	7·0
(*c*) 4°	1	1.7	89	12·3	7·2
	2	1.10	88	12·3	7·2
	3	1·16	96	12·1	7·9
	4	1.20	93	12·2	7·6
	5	1.23	96	12·2	7·9
	6	1.27	101	12·8	7·9

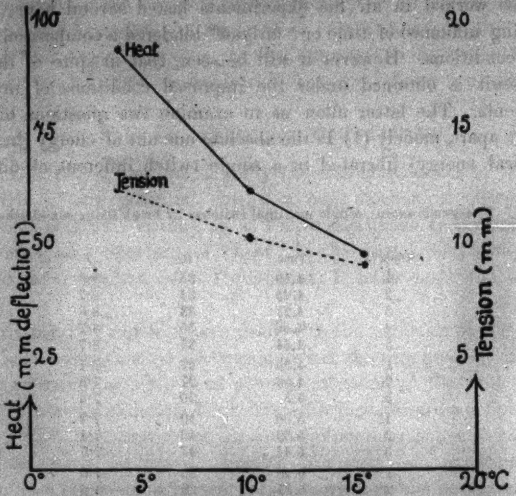


Fig. 2. Influence of temperature upon production of heat and tension in single twitches. (Exp. 2.)

Exp. 3. *Semimembranosus.* The experiment was interrupted at 12.44 and continued 6 hours later. There was a comparatively small decrease of H and T, but both kept constant during the last part of the experiment. Initial tension 2·2.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) 4·5°	1	11.42	122	13·8	8·8
	2	11.47	121	14·0	8·6
	3	11.50	119	14·1	8·4
	4	11.53	119	14·3	8·3
	5	11.58	117	14·4	8·1
(*b*) 16°	1	12.34	40	9·8	4·1
	2	12.37	49	10·2	4·8
	3	12.41	45	9·8	4·6
	4	12.44	50	11·0	4·5
	5	6.23 p.m.	44	8·7	5·1
	6	6.29	44	8·9	4·9
	7	6.31	43	8·8	4·9
(*c*) 8°	1	6.59	67	11·2	6·0
	2	7.3	67	11·5	5·8
	3	7.6	68	11·4	6·0
	4	7.9	68	11·3	6·0
	5	7.13	67	11·4	5·9
(*d*) 16°	1	7.39	41	8·5	4·8
	2	7.44	40	8·7	4·6
	3	7.47	39	8·7	4·5
	4	7.51	41	8·7	4·8
	5	7.53	38	8·3	4·6

Exp. 4. *Semimembranosus.*

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) 15°	1	11.25	42	7·8	5·4
	2	11.28	38	7·6	5·0
	3	11.33	39	7·7	5·1
	4	11.36	39	7·6	5·1
	5	11.39	40	7·6	5·3
(*b*) 5°	1	12.6	99	9·8	10·1
	2	12.8	94	10·0	9·4
	3	12.13	102	9·7	10·7
	4	12.15	101	9·5	10·6
	5	12.17	99	9·3	10·6
	6	12.19	104	8·8	11·8
	7	12.29	116	8·3	14·0

These four experiments, which were perhaps the best in a total number of 14 or 15, show that the average error is scarcely increased by the change of temperature in the Dewar-flask.

temperatures, and in what way? (2) Is the proportion between heat and mechanical energy different at different temperatures and in what way?

In accordance with results obtained by Bernstein[1] and Fröhlich[2] I find in most cases a more or less marked increase of the twitches at temperature lower than 18°; the lower the temperature, the higher the twitch. I also find that the heat-production is always very much higher, the lower the temperature is. The heat-production being increased even more than the tension set up, the ratio H/T is likewise increased at lower temperatures. If we suppose that T is proportional to the maximum work, we may also conclude that the "efficiency" is smaller at lower temperatures.

This result is absolutely the same in all my experiments except in two. These were the first ones; I made and had several technical mistakes, the average error also was much higher. In these experiments there was no difference of average heat-production or of tension at different temperatures, the temp. coefficient being = 1. But the figures were rather irregular, and I do not think that we are compelled to assume "individual" peculiarities in these cases, but merely errors. The following table T will show that the "temperature coefficients" in the other experiments show fair accordance. Nevertheless the accordance is not as good as one might expect from the very constant results in one and the same experiment.

Since there is a decrease of heat-production while the temperature rises, the temp. coefficient is below 1. Its average value is 0·63, *i.e.* the heat-production drops at an increase of 10° about 40 %, while the "efficiency" *T* rises at the same time approximately in the same degree or somewhat less.

---

[1] Bernstein. *Pflüger's Arch.* CXXII. pp. 124, 129, 462.

[2] Fröhlich. *Ibid.* CXXIII. p. 696; *Ztschr. f. allg. Physiol.* VII. p. 461.

TABLE II.

Exp.	Temperature	Initial heat-production	Tension	$\frac{\text{Heat}}{\text{Tension}}$	Temp. coeff. of heat-prod. for 10° C.
1	4°	128	11·7	10·9	—
	16°	86	12·1	7·1	0·71
2	5°	99	13·4	7·4	—
	17°	104	13·0	8·0	1·0
3	25·5°	104	12·6	8·2	—
	14·5°	102	12·0	8·5	1·0
4	4°	111	12·1	9·2	—
	14°	70	13·5	5·2	0·68
5	16°	91	16·3	5·6	—
	4°	152	16·7	9·1	0·64
	18°	110	18·9	5·8	0·70
6	4·5°	120	14·1	8·5	—
	16·0°	45	9·6	4·7	0·41
	8·0°	67	11·4	5·9	0·62
	16·0°	40	8·6	4·7	0·54
7	15°	47	9·2	5·1	—
	10°	72	10·4	6·9	0·49
	4°	94	12·2	7·7	0·66
8	15°	39	7·7	5·1	—
	5°	102	9·3	11·0	0·38
9	8·0°	25	7·7	3·3	—
	16·5°	11	5·4	2·0	0·41
	11·5°	15	6·0	2·5	0·58
10	15°	39	8·5	4·6	—
	21°	25	6·5	3·8	0·52
	16°	34	6·6	5·1	0·59
11	16·0°	34	8·7	4·0	—
	22·5°	27	7·5	3·5	0·71
	14·5°	42	7·4	5·6	0·59
	25·5°	28	6·9	4·1	0·68
	12·0°	48	8·0	6·0	0·65

The "temperature coefficient" for 10° was calculated as follows. If *e.g.* the heat-production was 100 scale-divisions at 5° and 70 at 12°, the difference being 30 for 7°, I regarded the difference for 1° as 30 ÷ 7 = 4·3 and for 10° as 4·3 × 10 = 43. I thus assumed a linear function and not an exponential-function as Meyerhof[1] and Warburg[2] have done. It must be said, that the expression of "temperature coefficient" covers in biology at present a ground quite different from any thermo-chemical conception.

In this actual case of course any idea of application of the so-called rule of van t' Hoff is excluded from the beginning, since the reaction is checked by rising temperatures, and the total heat-production does not represent a velocity at all.

---

[1] *Pflüger's Arch.* CXLVI. p. 159. 1912.

[2] *Ergebn. d. Physiol.* XIV. p. 313. 1914.

Some other experiments have been carried out on short isometric tetanus. Although they have been less satisfactory, they gave clear enough evidence. Several difficulties join to impair these experiments. The muscles are very much injured by a series of even short tetani. It is necessary however to use maximal stimuli, in order to ensure constancy. But the maximum tetanus is reached by currents much weaker than those which are necessary for the maximum heat-production. It needs 3 or 4 observations to find out the proper strengths of stimulation. It is not easy to find the right economy in getting a sufficient number of observations with as few stimuli as possible.

In most cases the height of contraction was higher at high, lower at low temperatures—in contrast to the single twitch.

Exp. 1. *Semimembranosus.* Tetanus during 0·5 sec. in each observation. Current-strengths sufficient to produce a maximum heat-production.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) 15° C.	1	10.52	208	20·2	10·3
	2	10.56	204	19·8	10·3
	3	11.0	200	19·0	10·5
	4	11.6	190	18·6	10·2
(*b*) 3·5°	1	11.36	168	16·0	10·2
	2	11.41	169	16·0	10·6
	3	11.45	166	15·4	10·8
	4	11.49	164	15·7	10·4
	5	11.55	164	16·1	10·2
	6	12.0	175	16·2	10·8
(*c*) 15·0°	1	12.27	178	18·7	9·5
	2	12.43	172	17·0	10·1
	3	12.45	179	17·0	10·6
	4	12.48	169	16·4	10·3
	5	12.52	157	16·8	9·3
	6	12.56	156	15·7	9·9
(*d*) 4·0°	1	1.23	139	13·5	10·3
	2	1.27	133	12·8	10·4
	3	1.30	137	12·5	10·9
	4	1.33	142	13·5	10·5
	5	1.36	141	13·1	10·8

Exp. 2. *Semimembranosus.* 0·5 sec. tetanus.

Period	Observ.	Time	H	T	$\frac{H}{T}$
(*a*) 9·5° C.	1	10.48	243	15·0	16·2
	2	10.52	233	15·0	15·5
	3	11.10	233	13·5	17·2
(*b*) 2°	1	11.30	152	12·4	12·3
	2	11.37	169	12·3	13·8
	3	11.42	172	12·2	14·1
	4	11.49	189	12·8	14·8
	5	11.54	190	12·6	15·1
	6	11.57	187	12·5	15·0
	7	12.2	196	12·3	15·9
(*c*) 13·5°	1	12.24	191	17·0	11·2
	2	12.28	186	16·6	11·2
	3	12.35	183	16·3	11·2
	4	12.45	186	16·8	11·1

Period	Observ.	Time	*H*	*T*	$\frac{H}{T}$
(*d*) 2°	1	1.3	133	10·7	12·4
	2	1.7	117	10·7	11·0
	3	1.13	136	10·0	13·6
	4	1.15	133	10·0	13·3
(*e*) 14°	1	1.32	170	15·2	11·2
	2	1.34	205	15·0	13·7
	3	1.37	155	15·2	10·2
	4	1.40	158	14·6	10·8
	5	1.43	157	14·5	10·8
	6	1.48	167	14·2	11·8
	7	1.52	148	14·0	10·6

Exp. 3. *Semimembranosus.* 0·5 sec. tetanus.

(*a*) 15° C.	1	6.27	165	25·5	6·5
	2	6.32	165	20·8	7·9
	3	6.37	169	20·6	8·2
	4	6.43	168	19·0	8·8
(*b*) 3°	1	7.5	95	14·0	6·8
	2	7.17	93	13·0	7·2
	3	7.22	77	10·2	7·5
	4	7.25	90	10·2	8·8
	5	7.31	92	10·1	9·1
(*c*) 13°	1	7.52	134	14·4	9·3
	2	7.56	128	14·8	8·6
	3	8.5	118	13·8	8·5
	4	8.8	115	13·3	8·6

These three experiments (and five other similar experiments, see p. 403) also show, although the figures are less satisfactory than in all the other experiments and "fatigue" is going on, that there exists a marked difference from the results with single twitches. The absolute value of the initial heat in tetanus is either the same at different temperatures, or it is smaller at lower, larger at higher temperatures (*e.g.* Exp. 2, period *c*, *d*, *e*, and Exp. 3), the result being directly opposite to the behaviour of single twitches.

The explanation is probably very simple. It is very likely that at a higher temperature a greater number of tetanising stimuli are really effective, because the refractory period is shorter and the whole contraction-process going on quicker. From this we should expect a greater heat-production in a tetanus of a given time at higher than at lower temperature, simply because the number of effective stimuli is different. But this effect will be counteracted by the fact, which has been shown above, that the heat produced by one single excitatory process is smaller at higher temperatures. The result will be that this decrease at high temperature is balanced or even overbalanced by the greater number of excitatory processes.

In the general Table III of all the experiments on tetanus no temperature-coefficient of initial heat-production has been calculated. A calculation of the temperature-coefficient of heat-production for a given tension $\left(\frac{H}{T}\right)$ gives an average value of 1·0. The average "efficiency" of the tetanised muscle, and probably also of voluntarily contracting muscle, is therefore almost independent of temperature.

It should distinctly be understood that all the results given in this part concern the temperatures between 0° and 22°. Further investigation will be needed for the range between 20° and 35° (p. 400). Also the difference between isometric and isotonic twitches, suggested by Fick, is an open question.

PART III. EFFECT OF DRUGS.

Many facts concerning the effect of drugs etc. which will be described here, have only been detected because this method allows of following the events from minute to minute. While gas-analyses always give a summary or average of periods of a certain duration the myothermic experiment takes things to pieces from moment to moment. By a proper management it is possible to observe the heat-production as early as one or two minutes after we have added a certain substance to the solution. It is probable that a great many substances have a different influence while they are passing through the surface of cells and when equilibrium between the cell and the *milieu* is obtained. These and other instances of short-duration effects can be studied without difficulty.

1. *Alcohol.*

The effects of alcohol are complex and cannot be treated here exhaustively. The main features will be seen from the experiments[1] which follow. The first effect of a concentration of 3 or 4 °/₀ ethyl-alcohol is a very marked increase of initial heat-production, which may last about ten minutes. This may be accompanied by a slight increase of production of tension, but not necessarily.

---

[1] Six other experiments are not reported, because they gave exactly the same results.

TABLE III.

Exp.	Temperature	Initial heat-production	Tension	$\frac{\text{Heat}}{\text{Tension}}$	Temp. coeff. of H/T for 10°
1	3°	474	16·5	28·8	—
	15°	400	16·9	23·6	0·85
2	15°	201	19·5	10·3	—
	3·5°	167	15·9	10·5	1·0
	15°	169	17·0	9·9	0·95
	4°	138	13·0	10·6	0·95
3	7°	238	29·0	8·2	—
	17·5°	241	23·2	10·4	1·26
	3°	136	17·0	8·0	1·20
4	3·5°	81	7·6	10·6	—
	14°	100	12·5	8·0	0·76
5	5°	107	15·5	6·9	—
	15°	126	13·8	9·1	1·32
	15°	99	11·6	8·6	—
	9·5°	84	10·2	8·2	1·05
6	9·5°	237	14·5	16·3	—
	2·0°	176	12·2	14·4	1·18
	13·5°	187	16·7	11·2	0·80
	2·0°	130	10·3	12·6	0·90
	14·0°	166	14·7	11·3	0·91
7	15°	166	21·0	7·9	—
	3°	89	11·2	7·9	1·00
	13°	124	14·1	8·8	1·11
8	3°	206	10·8	19·1	—
	15°	291	14·1	20·7	1·07
	5°	262	10·9	24·0	0·86
	15°	251	13·0	19·3	0·80
9	3·5°	250	7·5	33·5	—
	16°	223	11·3	19·7	0·64
	25·5°	254	8·8	28·9	1·48
	16°	185	6·1	30·4	0·95

EXP. 1. *Semimembranosus.* (2 × 170 mg.)

	Observ.	Time	H	T	$\frac{H}{T}$
Ringer's fluid	1	4.55	33	10·9	3·0
	2	4.57	35	10·8	3·2
	3	4.59	35	11·0	3·2
	4	5.1	35	11·0	3·2
3 % alcohol at 5.5	1	5.43	33	7·3	4·5
	2	5.47	31	7·0	4·4
	3	5.49	30	7·1	4·2
	4	5.51	28	7·1	3·9
Ringer	1	6.30	34	10·1	3·4
	2	6.30	34	9·0	3·8
	3	6.30	28	9·0	3·1
	4	6.30	33	10·2	3·2
	5	6.30	36	10·6	3·4

EXP. 2. *Semimembranosus.* (2 × 195 mg.)

	Observ.	Time	H	T	$\frac{H}{T}$
Ringer	1	10.36	72	14·9	4·8
	2	10.41	73	14·5	5·0
	3	10.44	73	14·4	5·0
	4	10.50	70	14·1	5·0
3·1 % alcohol at 10.55	1	11.29	57	10·6	5·4
	2	11.32	53	10·8	4·9
	3	11.35	52	10·0	5·2
	4	11.42	71	11·8	6·0
	5	11.46	62	9·9	6·3

	Observ.	Time	H	T	$\frac{H}{T}$
4·5 °/₀ alcohol at 11.50	1	1.6	19	1·1	17·2
	2	1.9	9	0·9	10·0
	3	1.18	11	0·3	36·6
	4	1.22	12	0·6	20·0
	5	3.52	10	0·2	50·0
Ringer	1	4.34	52	15·1	3·4
	2	4.49	58	14·5	4·0
	3	4.51	62	14·8	4·2

Exp. 3. *Semimembranosus.* Ethyl-alcohol. Temp. 14°. Maximum shocks.

Ringer solution	1	12.49	34	8·8	3·9
	2	12.53	33	8·8	3·7
	3	12.55	35	9·2	3·8
	4	12.58	34	9·0	3·8
	5	1.0	35	9·2	3·8
3 °/₀ alcohol at 1.5	1	1.14	33	8·5	3·9
	2	1.17	38	8·8	4·3
	3	1.21	39	8·3	4·7
	4	1.24	41	8·0	4·7
	5	1.29	37	7·5	4·9
	6	2.42	21	4·1	5·1
	7	2.47	20	4·1	4·9
	8	2.51	20	4·1	4·9
	9	2.55	20	4·1	4·9
4·5 °/₀ alcohol at 3 p.m.	1	3.10	20	3·1	6·4
	2	3.15	22	2·9	7·6
	3	3.21	22	2·7	8·2
	4	3.32	23	2·4	9·6
	5	3.35	23	2·0	11·5
	6	3.49	22	2·0	11·0
5·5 °/₀ alcohol	1	3.56	17	1·3	13·1
	2	3.59	16	1·0	16·0
	3	4.1	18	1·3	13·8
	4	4.3	14	1·2	11·6
6·0 °/₀ alcohol	1	4.14	11	0·7	15·7
	2	4.17	8	0·4	20·0
	3	4.21	9	0·3	30·0
0·002 *n* KCN	1	4.33	9	0·1	90·0
	2	4.39	6·5	0·0	0·0
	3	4.46	6·5	0·0	0·0
	4	4.57	6·5	0·0	0·0
Alcohol removed; pure Ringer-solution	1	5.14	10	1·0	10·0
	2	5.23	27	6·6	4·1
	3	5.34	55	10·3	5·3
	4	5.36	42	10·0	4·2
	5	5.44	44	10·5	4·2
	6	5.53	61	11·7	5·2
	7	5.59	71	12·0	5·9
	8	6.2	88	12·2	7·3
	9	6.5	68	11·2	6·1
	10	6.11	58	11·3	5·1
	11	6.17	30	7·8	3·9
	12	6.19	26	7·0	3·7
	13	6.21	24	6·9	3·5
	14	6.27	28	7·3	3·8
	15	6.34	24	6·9	3·5
	16	6.42	24	6·9	3·5

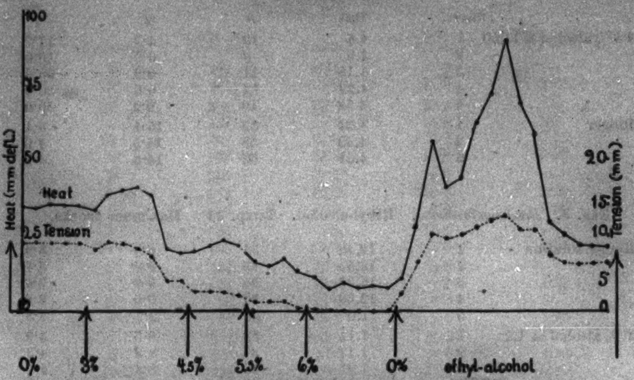


Fig. 3. Influence of *ethyl-alcohol* upon production of heat and tension in single twitches. (Exp. 3.)

Exp. 4. *Semimembranosus.* Conditions as before.

	Observ.	Time	H	T	$\frac{H}{T}$
Normal	1	10.17	43	7·9	5·4
	2	10.19	44	8·0	5·5
	3	10.21	45	7·9	5·7
	4	10.23	43	8·1	5·3
3·5 °/₀ ethyl-alcohol	1	10.34	56	8·4	6·7
	2	10.38	53	7·3	7·3
	3	10.40	50	6·6	7·6
	4	10.45	47	5·9	8·0
	5	10.50	43	5·3	8·1
	6	10.53	40	4·9	8·2
	7	10.58	41	4·8	8·5
	8	11.39	45	3·4	13·2
	9	11.45	31	3·4	9·1
	10	11.49	30	3·3	9·1
	11	11.51	29	3·3	8·8
4·5 °/₀ alcohol	1	11.59	39	3·3	11·8
	2	12.3	35	3·1	11·4
	3	12.14	36	2·6	13·8
	4	12.19	24	2·3	10·4
	5	12.25	22	2·3	9·6
	6	12.37	21	2·2	9·5
5·5 °/₀ alcohol	1	12.41	30	1·0	30·0
	2	12.45	18	0	∞
	3	12.47	9	0	∞
Normal Ringer's solution	1	1.5	23	4·1	5·6
	2	1.8	26	4·3	6·0
	3	1.16	30	5·6	5·4
	4	1.22	34	6·2	5·5
	5	1.32	36	6·4	5·6
	6	1.41	37	6·8	5·4
	7	1.46	37	6·8	5·4
	8	1.55	37	6·8	5·4

TABLE IV.

Exp.		*H*	*T*
1.	Normal	28	6·1
	Alcohol	15	0
2.	Normal	34	9·0
	Alcohol	6·5	0
3.	Normal	44	8·0
	Alcohol	18	0
4.	Normal	85	11·5
	Alcohol	21	0

Exps. 1 and 2 are representatives of a series carried out with a less refined method. I had then to wait between two "periods" about 30 minutes. When observations began the muscle was therefore in equilibrium with the alcohol. Different from this is the type of experiments represented by Exps. 3 and 4. Observation began a few minutes after a new condition had been effected. It was only in these experiments that the effects of alcohol in increasing the heat-output could be detected, because these effects pass off so quickly. Heat and tension then drop to a certain level, which remains more or less constant. Further increase of alcohol-concentration produced again not seldom a passing increase of heat-production (*e.g.* Exp. 4). While smaller concentrations often produce a parallel decrease of heat and tension (*e.g.* Exp. 2 with 3·1 %, alcohol), the concentrations from 4 %–6 % have always a preponderating influence upon the mechanical response; when the mechanical response is completely extinguished each stimulus has still the effect of heat-liberation, the deflections now being 20 %–50 % of their normal value (Table IV). The excitation of muscle therefore results now in heat-production without any transformation of energy into mechanical energy. The deflections of the galvanometer seemed sometimes to stay longer on their maximum value than usual. These effects of alcohol are *reversible.* When the alcohol is removed the muscle recovers and returns to the normal condition. When the contractility had been absolutely abolished the recovery failed sometimes to be quite complete, the muscle however reaching always more than 60 or 70 % of its normal heat-production and tension-production.

The fact that at high concentrations of alcohol the "efficiency" of muscle is lowered more and more, recalls experiments which have been carried out by Rohde and Ogawa[1] on cats' hearts with chloral-hydrate. They show a marked decrease of the proportion between pressure developed in an isometric twitch and O_2-consumption. I have found the same fact for the frog's heart and have shown at the same time that the action of alcohol must be due to an alteration of the contractile machine and not only of the oxidative part of the system[1]. This statement is justified by the results shown above.

---

[1] E. Rohde and Ogawa. *Arch. f. exp. Path. u. Pharmak.* LXIX. p. 214. 1912.

It is not unlikely that two effects of alcohol must be distinguished. We have seen above that the immediate effect of increasing the concentration is increase of energy-production. The same effect can be observed while the muscle returns from narcosis to normal conditions in pure Ringer solution. Exp. 2 shows an increase of more than 100 °/₀ over the normal value. The effect therefore occurs when a *change of concentration* occurs outside the cell, equally, be it increase or decrease. It seems to me not unlikely that it is due to the *movement* of alcohol through the surface of cells. The existence of this kind of influence has been shown at first by W. Straub (with muscarine)[2], but the alcohol and similar substances have generally not been regarded as belonging to this type of "potential gift." But there is no reason why this drug should not have "potential" effects as well as effects of combination. By entering or leaving it has an exciting effect, by staying in equilibrium it has a checking effect.

Another explanation would be that small concentrations are exciting, but great ones checking. The explanation has been abandoned, because I never could obtain any persisting action of the former kind. Small concentrations have, as is seen from the tables, a small checking effect or no effect at all, and exciting effects occur rather more with strong concentrations.

The increase of heat-production by alcohol entering or leaving the cell and the heat-production without contraction are—although the effects are reversible—perhaps the first stage of what happens in wider range during contracture and rigor. This state of muscle is characterised by a disposition for discharging quite abnormal quantities of energy after a stimulus. This will be shown later as I hope also in the case of caffeine.

The opinion that the change of concentrations (inside or outside the cells) is responsible for abnormal discharge of energy is supported by my observation that these effects appear more distinctly if suddenly a strong change is effected, and are less obvious when a change happens gradually. The most obvious check of energy transformation was observed when suddenly higher concentrations of alcohol were applied.

---

[1] Weizsäcker, *l.c.*

[2] W. Straub. *Pflüger's Arch.* CXIX. p. 127. 1907.

2. *Hypotonic solutions.*

The effect of hypotonic solutions (see Exps. below) show a remarkable difference from the effect just described. No increase of energy-liberation has been observed. But the checking effect on production of heat and tension is about equal. Both become less and less, and they disappear at about the same time, the proportion H/T being probably slightly increased at the last stage of activity. In most experiments the mechanical response vanished when the heat-production was about 10 % or less of its normal value. Near zero the errors however naturally grow very much, and as a whole we can say that *the efficiency* of the contraction machine as far as production of tension is concerned is only slightly altered by hypotonic solutions (see Fig. 4). Also these effects are *reversible.*

The "water rigor" of muscle is therefore a thing entirely different from the chemical rigors and includes no disposition for abnormal break-down. The weight of the muscles must have been increased by about 50 %[1], since I used solution containing ½, ⅓, or even ¼ of their usual salt-concentration.

My experiments on frog's-heart[2] have shown that the oxidation in such cases is checked in about the same degree as the mechanical work. Now Siebeck[3] has shown that the oxidation occurring in resting cells (the kidneys of frogs) is unaltered when the organ is kept in hypotonic solutions, although their weight increases as well as the weight of muscles. We must conclude that the mechanism of oxidation, considered by itself, is not checked by hypotony, and so we are forced to the further conclusion that if it is diminished in the beating heart, the diminution is due to the action upon the contractile mechanism. It follows that the oxidation in the active muscle depends upon the action of the contractile mechanism and cannot be started without activity of the contracting part of the system.

The influence of an increased water-content of muscle cells is therefore chiefly a decrease of discharge of the energy which produces mechanical energy. Unfortunately no investigation of the action current in this case seems to exist. But it is likely that the action current is going on, even when no contraction appears, because

---

[1] **Overton.** *Pflüger's Arch.* XCII. 1902; CV. 1904.

[2] **Weizsäcker.** *Naturhistor.-med. Ver.* Heidelberg, *l.c.*

[3] **Siebeck.** *Pflüger's Arch.* CXLVIII. pp. 473 and 504. 1912.

Biedermann[1] and after him Overton[2] have shown that both the excitability, and the conductivity of the excitatory process, are conserved in such muscles. I hope to come back to the question at another time. Similar problems of course arise for the muscles in alcohol.

Exp. 1. *Semimembranosus.*

Period	Observ.	Time	H	T	$\frac{H}{T}$
(a)	1	10.0	46	10·8	4·3
	2	10.5	45	10·8	4·2
	3	10·9	47	10·9	4·3
	4	10.13	47	10·9	4·3
	5	10.16	47	10·8	4·3
(b) ½ isotonic	1	10.33	46	9·4	4·9
	2	10.37	42	9·0	4·7
	3	10.41	40	8·8	4·5
	4	10.49	36	7·9	4·6
	5	10.53	35	7·8	4·5
	6	10.57	34	7·3	4·7
	7	11.3	32	6·2	5·2
	8	11.10	28	6·1	4·6
	9	11.15	28	5·3	5·3
	10	11.32	26	4·6	5·6
	11	11.46	21	4·3	4·9
	12	11.50	20	4·1	4·9
	13	11.59	17	3·9	4·4
	14	12.8	16	3·9	4·1
	15	12.14	17	3·8	4·5
	16	12.22	17	3·8	4·5
	17	12.25	15	3·7	4·1
(c) ¼ isotonic	1	12.40	12	3·2	3·8
	2	12.44	12	2·8	4·3
	3	12.47	12	2·7	4·5
	4	12.50	13	2·4	5·4
	5	12.55	9	2·1	4·3
	6	12.59	9	1·6	5·6
	7	1.3	8	1·2	6·7
	8	1.7	6	0·9	6·7
	9	1.10	6	0·7	8·5
	10	1.13	6	0·6	10·0
	11	1.17	5	—	—
	12	1.20	4	—	—
(d)	1	3.6	25	7·4	3·4
	2	3.9	25	7·7	3·3
	3	3.26	26	7·7	3·4
	4	3.31	27	7·7	3·5
	5	3.34	27	7·8	3·5

---

[1] **Biedermann.** *Electro-Physiology*, I. p. 164. Transl. by **Welby.** London, 1896. *Wiener Stzngsb.* XCVII. III. 1888.

[2] **Overton.** *Pflüger's Arch.* XCII. p. 146. 1902.

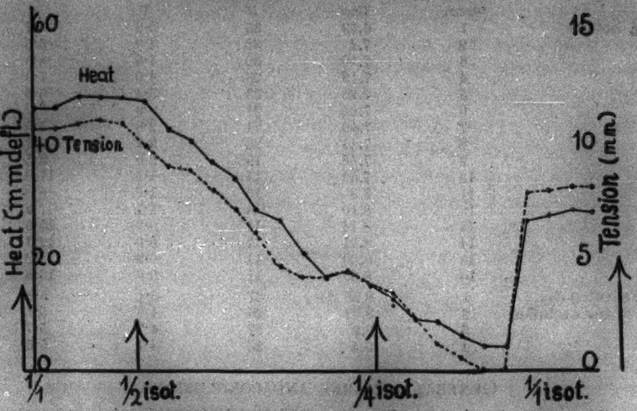


Fig. 4. Influence of *hypotonic Ringer's-solution* upon production of heat and tension in single twitches. (Exp. 1.)

Exp. 2. *Semimembranosus.*

	Observ.	Time	*H*	*T*	$\frac{H}{T}$
	1	11.24	28	6·1	4·6
	2	11.26	30	6·2	4·8
	3	11.29	31	6·6	4·7
	4	11.32	32	6·8	4·7
	5	11.34	25	6·2	4·0
	6	11.43	29	6·3	4·6
	7	11.47	30	6·2	4·8
½ isotonic	1	12.3	24	4·8	5·0
	2	12.14	21	4·5	4·7
	3	12.31	21	4·0	5·2
	4	12.33	19	4·0	4·8
	5	12.47	16	3·7	4·3
	6	12.50	19	3·7	5·1
	7	12.59	16	3·2	5·0
Ringer	1	4.24	3	0·8	3·8
	2	4.25	3	1·0	3·0
	3	4.26	3	0·9	3·3
	4	4.30	4	1·4	2·5
	5	4.36	4	1·2	3·3
	6	4.43	6	1·4	4·3
	7	4.52	7	1·8	3·9
	8	4.55	5	2·2	2·3
	9	4.59	6	2·2	2·7
	10	5.3	7	2·4	2·9
	11	5.3	7	2·4	2·9

Exp. 3. *Semimembranosus.*

	Observ.	Time	*H*	*T*	$\frac{H}{T}$
	1	6.37	41	6·8	6·0
	2	6.38	41	7·0	5·9
	3	6.40	42	6·8	6·2
	4	6.42	42	7·0	6·0

	Observ.	Time	H	T	$\frac{H}{T}$
½ isotonic	1	6.59	35	5·2	6·7
	2	7.4	32	4·9	6·5
	3	7.10	30	4·6	6·5
	4	7.19	28	4·0	7·0
	5	7.29	23	3·4	6·8
	6	7.35	21	2·9	7·2
	7	7.40	21	2·8	7·5
	8	7.44	20	2·2	8·9
	9	7.46	19	2·0	9·5
	10	7.59	17	1·5	11·0
	11	8.3	15	1·7	8·8
	12	8.10	14	1·7	7·9
	13	8.14	14	0·9	15·6
	14	8.21	12	0·9	12·3
	15	8.30	11	0·9	12·2
	16	8.39	11	0·8	13·8
Following day, Ringer's solution	1	8.55	27	5·1	5·3
	2	8.57	26	4·8	5·4
	3	9.2	27	4·7	5·7
	4	9.4	26	4·7	5·5

GENERAL REMARKS AND CONCLUSIONS.

The general point of view from which this investigation starts has been put forward above. A. V. Hill's experiments and the evidence given in Part I show that it is possible to separate from the whole chain of processes going on in an active muscle the special part which is immediately concerned with the production of mechanical energy, and it shows that this part involves no oxidation. Therefore it is possible to study separately the contractile mechanism on the one hand and the recovery mechanism on the other.

The prominent feature of the contractile mechanism is undoubtedly its behaviour at different temperatures. No physical or chemical phenomenon of elementary nature is accelerated at lower temperature. It is therefore unthinkable that the influence of temperature on the discharge of energy in muscle has anything to do with the velocity of any elementary process such as chemical reaction, diffusion, etc. But it is conceivable that the condition of some complicated physico-chemical system, or link of such system, is responsible for the facts shown in Part II. In this case the facts are not necessarily surprising. If *e.g.* the degree of dispersity in a colloidal system is diminished by higher temperature the active surface becomes smaller, and a catalytic reaction, which is in connection with this surface, will therefore be decreased. But Mr A. V. Hill has suggested to me that possibly a purely thermodynamical explanation of this phenomenon may be sufficient. Let us suppose that contraction is due to some balanced reaction of the type

$$AL \rightleftarrows A + L$$

in which (say) lactic acid is liberated from some precursor until (for the moment and before neutralisation and diffusion have taken place) an equilibrium is reached: the reaction is known to go on with an evolution of heat: and therefore according to Le Chatelier's rule the equilibrium is forced ← by a rise of temperature, → by a fall of temperature, in other words a rise of temperature diminishes the response. These are only two of many possible explanations, but any theory of contraction will have to bear this effect of temperature in mind.

Also from the standpoint of practical biology it is remarkable that the muscle of the cold-blooded animal is able to afford work independent within wide limits of the general checking influence of low temperatures. The frog's mobility is—as far as the muscle is concerned—in no way impaired by the cold. This property to some extent counterbalances the natural advantage of the warm-blooded animal. Further, in tetanus the heat and the mechanical response seem also to be largely unaffected by temperature.

Here we may recall that the frog has an advantage over the mammal in the effect of lack of oxygen upon the heart. **Rohde**[1] has shown that KCN diminishes the isometric twitches of a cat's heart even more than its oxygen-consumption, while I[2] have found that the same drug allows the frog's heart-beat to go on for a long time even if oxidation has been completely stopped.

Let us consider now more closely the contractile mechanism. The opinion that we can stop the production of mechanical energy without stopping various other chemical and physical phenomena is already supported by other observations. The action current, to begin with, is certainly independent of mechanical action; it goes on in a heart brought to rest by several influences (**Locke**, **Noyons**, **Seemann**, **Mines**). I have found also in some unpublished experiments that the action current is unaltered when the oxidations are completely stopped by cyanide. In the next place it is shown by **Locke** and **Rosenheim**[3] and by **Rohde**[4] that the disappearance of sugar can go on in hearts which beat only weakly or not at all. This of course only proves that sugar goes to the cells, not that it is destroyed, or that energy is liberated. Further, **Rohde**[5] has found that about $\frac{2}{3}$ of the oxidation may be going on whilst the mechanical response is nearly extinguished by CO_2. Similar observations are already reported by Locke and Rosenheim, who measured the yield of CO_2.

---

[1] **Rohde.** *Arch. f. exp. Path. u. Pharm., l.c.*

[2] **Weizsäcker.** *Pflüger's Arch.* CXLVII. p. 185. 1912.

[3] **Locke** and **Rosenheim.** This *Journal*, XXXVI. p. 205. 1907.

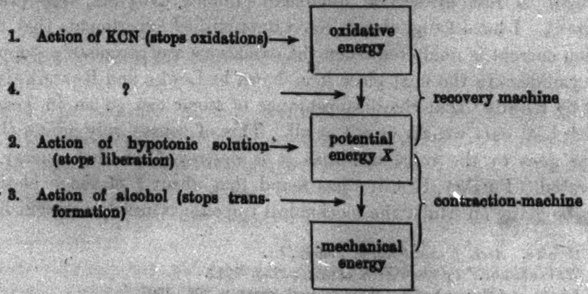
[4] **Rohde.** *Zntrlb. f. Physiol.*

[5] **Rohde.** *Ibid.* XXVII. No. 21.

Since the initial heat-production has nothing to do with oxidation it is an entirely different question, whether we can stop the twitch without stopping the initial heat. As I have shown above, this is possible. Stronger concentrations of alcohol produce a complete but reversible block in the transformation of energy into mechanical energy. We must therefore assume that the substance which provides energy is different and works to some extent independently of the contractile substance.

It is of some interest also for the theory of narcosis that alcohol influences most of all the contractile substance. In a previous communication I[1] showed that alcohol works in the first place on the contractile mechanism and only in much higher concentrations also upon the oxidations. This opinion is confirmed by this paper and in addition it has been found that most of all it works upon the actual contractile bodies of the contraction-mechanism.

The statement that there is a particular point in the mechanism where it is possible to establish a "block" between the energy providing and the contractile apparatus is supported by the definite difference between this type of influence (alcohol) and the type which is represented by the hypotonic experiments in which there is a much smaller or no influence at all upon the transformation of energy. It is clear that when less energy is discharged in the providing part also less energy will be transformed into mechanical energy. The complex chain of phenomena and the different influences upon them may perhaps be better appreciated with the aid of the following scheme:


1. Action of KCN (stops oxidations)
oxidative energy
4. ?
recovery machine
2. Action of hypotonic solution (stops liberation)
potential energy X
3. Action of alcohol (stops transformation)
contraction-machine
mechanical energy

---

[1] Weizsäcker. *Naturhistor. med. Verein*, Heidelberg, *l.c.*

The two perpendicular arrows signify the transformation of one energy into another, the horizontal arrows simply point out upon what stage of the whole process the different influences are working. The main facts which support this picture have been mentioned above. There are three types of influences described in this paper:

1. Stopping the oxidations (*a*) by lack of oxygen, (*b*) by cyanide.
2. Influencing the liberation of the potential energy *X*, which liberation is (*a*) increased by lowering the temperature, (*b*) decreased by hypotonic solutions (and alcohol).
3. Influencing the transformation of *X* into mechanical energy (the "efficiency"): (*a*) improving the efficiency by raising the temperature, (*b*) lowering the efficiency by the higher concentrations of alcohol.

But there is a fourth possibility of interest not yet considered, namely, the transformation of oxidative energy into the potential energy *X*. This is a chapter of the thermodynamics of muscle which has never been touched as yet. But I may be allowed to describe some facts which may conduce to further insight. In a previous paper I have shown[1] that the oxidations in the beating frog's heart are increased, the higher the temperature. In the present paper I show that the initial heat behaves exactly in the opposite fashion. If we are not to assume that the heart and the semimembranosus muscle have different thermodynamic relations, we must conclude that the efficiency of the recovery machine is worse at higher temperatures, while that of the contraction machine is better. I am aware that further evidence is needed before we can definitely draw such a conclusion, but the conclusion is by no means unlikely. The so-called "recovery heat-production" (Hill) is proof enough that the recovery machine works with considerable losses of energy. These losses now seem to be increased very much by heating the muscle, but may be counterbalanced more or less by the better efficiency of the contractile mechanism.

On this line we may hope to advance to the thermodynamic properties of the recovery machine. But the purely "thermodynamic" consideration leads us to further problems, especially to the problem, by what mechanism the discharge of energy may be produced. At present our conclusions concerning this mechanism of *energy-discharge* may go as far as the following statement. The first point for a natural or artificial stimulus to act upon is a certain mechanism which is able to discharge a certain amount of non-oxidative energy, stored up in a form not yet known (*e.g.* as glycogen or sugar). The free energy of the reaction when liberated can, but need not, be transformed into mechanical energy: under certain conditions (*e.g.* alcohol) it may appear merely as heat. The efficiency of the transformation can be influenced by temperature and by certain drugs, but not by oxygen pressure; also the extent of the total discharge depends on the temperature and on various chemical conditions, but not on oxygen pressure. This discharge starts a second slower transformation of energy, namely, an oxidation. The free energy of the oxidised bodies can be transformed into a certain potential energy, stored up as mentioned above. The amount of oxidative breakdown depends chiefly on the amount of the first, the non-oxidative discharge. But it is not unlikely that several conditions act in different ways upon the two reactions and upon the efficiencies of the two machines. Nevertheless the extent of the oxidations probably shows as a rule close parallelism with the discharge of initial heat, a conclusion which can be drawn from many comparisons between the work done by Rohde and myself on heart-muscles, and the myothermic experiments.

---

[1] **Weizsäcker.** *Pflüger's Arch.* CXLVIII. p. 535. 1912. The heart was artificially stimulated throughout all the different temperatures. The mechanical conditions were equally constant.

SUMMARY.

1. A method of carrying out myothermic experiments in salt-solutions is described. By this it is possible to obtain deflections of 100 mm. for a single twitch. This method has allowed one to investigate the influence of temperature and of various physico-chemical and chemical conditions.

2. The "initial heat-production" (A. V. Hill, total deflection of galvanometer) is the same in the presence or the absence of oxidations, whether oxygen be removed by nitrogen, or the oxidations be stopped by cyanide. The "initial heat-production" can therefore be regarded as entirely due to a non-oxidative process.

3. By raising the temperature from 1°–22° the initial heat-production of a single twitch is decreased about 40 °/ₒ for 10° C. Also the tension developed in an isometric twitch is slightly decreased, but in a much smaller degree. The average initial heat-production in a short tetanus (0·5 sec.) is for a given tension the same at different temperatures.

4. Ethyl-alcohol in small concentrations decreases the initial heat-production, and the tension developed in an isometric twitch in about the same degree. In higher concentrations it stops the production of

mechanical energy completely, whilst one-third and more of initial heat-production still takes place.

5. Hypotonic Ringer's solutions produce a parallel decrease of both heat and tension development, until both vanish at about the same time.

6. All these influences are reversible.

7. The results strengthen the theory of a chain of two different machines in muscle, (1) contraction-mechanism, (2) oxidative recovery mechanism. It is pointed out that a separate investigation of the thermodynamics of them both is necessary and possible. In this paper I have considered only the contraction-mechanism. It is shown that this is non-oxidative in nature and must be regarded as consisting of at least two parts, a part which provides energy and a part which transforms this into mechanical energy: the former can be active without the latter. It is shown that by various influences we can work upon at least three different points of the chain of processes going on in an active muscle.

I wish to express my respectful thanks to Prof. Langley for the great hospitality I have enjoyed in his laboratory. Most of all I am indebted to Mr A. V. Hill, who has allowed me to use his apparatus, and whose very kind help and advice have made this work to me a source of pleasure and instruction.

The expenses of the research have been borne in part by a Grant from the Royal Society to Mr Hill.

GASEOUS EXCHANGE IN THE DECEREBRATE ANIMAL. BY CHARLES G. L. WOLF AND T. S. HELE.

(From the Institute for the Study of Animal Nutrition, School of Agriculture, and the Physiological Laboratory, Cambridge University.)

THE greater part of the experiments which have been designed to test heat production and gaseous exchange, has been done on the intact animal, but many of the problems which await solution are done with difficulty in this way, and Tangl and his pupils have developed the Zuntz-Geppert method for use with animals which are curarized[1].

In his paper on the work of the kidney and the specific dynamic action of protein Tangl has given some of the factors which are necessary to be controlled if one is to obtain exact comparison before and after the change which one is investigating. The influences of reflexes and muscular movement introduce uncontrollable errors and make the results of the experiments partially illusory, when the animal is not immobile. This is specially the case where differences amounting to less than 10 $^{\circ}/_{0}$ are sought for. In order to eliminate these adventitious factors Tangl introduced again the use of curarized animals which had previously been employed by Zuntz[2] and by Frank and Voit[3]. With this method Tangl reduced the muscular activity to the passive inflation of the lungs with a respiration pump, the cardiac activity, and the peristalsis of the intestinal tract.

While it is generally assumed that the curari method does not change the gaseous metabolism, it would seem of value if one had a method which gave the same motionless preparation, the metabolism of which could be investigated over a sufficient length of time to make comparison of the effects of various types of food material introduced intravenously or by the mouth. For this purpose the decerebrate animal offers an excellent opportunity, and so far as we are aware seems to have been used only by Roaf[1], who investigated the influence of decerebrate rigidity on the carbon dioxide output. Sections of peripheral nerves or curari did not have any effect on the respiratory exchange. In his first paper Roaf investigated the carbon dioxide output only. In a subsequent paper he took up the oxygen absorption and found that the abolition of rigidity lowered the amount of oxygen absorbed, giving a slight difference between the oxygen results and those for carbon dioxide. As he did not estimate the two factors at the one time, one is unable to ascertain from his work what was the effect of abolition of rigidity on the respiratory quotient, although one might infer that the quotient was raised. This might indicate that rigidity was being accomplished with a metabolism where relatively less carbohydrate was consumed, than when the animal was relaxed.

---

[1] *Biochem. Ztschr.* xxxiv. p. 1. 1911.

[2] *Arch. f. (Anat. u.) Physiol.* p. 38. 1884.

[3] *Ztschr. f. Biol.* xlii. p. 309. 1901.

While the investigation of the carbon dioxide output in the oxygen intake is valuable, considerably more information is available concerning intermediary metabolism when one has the amount of oxygen absorbed and the respiratory quotient in the same experiment. It seemed to us very desirable that in any investigation of the gaseous exchange in decerebrate animals both values should be examined at the same time.

The method used by Tangl for the analysis of air in his experiments was that of Zuntz-Geppert. This most excellent method, however, has some disadvantages. In the first place, the collection of the respired air over water can always lead to a certain amount of carbon dioxide loss, and what seems to be more serious from one point of view, the analysis must be made during the experiment, and an opportunity is only given for duplicate determinations. It seemed to us the better way to collect the samples of respired air over dry mercury and collect samples large enough so that three or four analyses could be made.

The analyses were performed in a Petterson-Tobiessen apparatus of the general type used by Jaquet[2], Stähelin[3] and by Grafe[4] in their metabolism experiments. The total content of the measuring pipette was 60 c.c. The head of the apparatus was of somewhat different form from that used by Jaquet, and as it has certain advantages it will be described. The absorption pipettes were attached to the horizontal cross arms (Fig. 1) and the measuring pipette to the lower vertical arm.

---

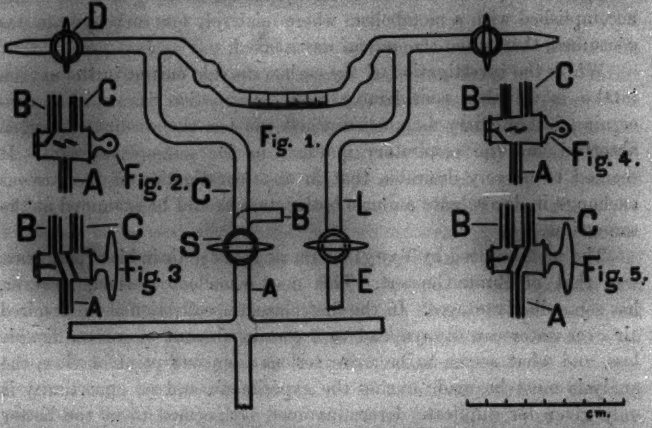
[1] *Quart. Journ. Exp. Physiol.* v. p. 31. 1912. *Ibid.* vi. p. 393. 1913. This *Journal*, XLIII. p. 449. 1912.

[2] *Verh. Naturf. Gesellsc.*, Basel, xv. p. 252. 1903.

[3] *Ibid.* xix. p. 107. 1904.

[4] *Ztschr. f. physiol. Chem.* LXV. p. 1. 1910.

Arm *A* carried a three-way stop-cock of the Greiner and Friedrich pattern (Figs. 2, 3, 4 and 5), one way of which communicated with the sampling tube through *B*, while the other communicated through *C* with the oil manometer familiar to those who have worked with the Petterson apparatus. This stop-cock had, however, another opening made by drilling a hole, horizontally, through the free end of the stopper and continuing this at right angles so that it communicated with the gas sampling extension. In this way, although the sampling tube is of convenient height from the base, one is in the position to fill the tube with the air to be sampled up to the stop-cock, and by turning the latter to make a communication immediately with the interior of the apparatus. The residual nitrogen is always expelled through the apparatus side of the manometer, so that before delivering a sample into the apparatus, all tubes on the measuring side of the apparatus are completely filled with nitrogen. The measuring tube was made under the supervision of one of us, and was graduated by weighing. A table was constructed giving the errors of the graduation. These errors for different parts of the scale were negligible.



Owing to the recent work of Benedict[1] one is in a position to judge of the accuracy of an apparatus for the analysis of air by the result it gives. From the results of 264 analyses of air, composed of samples from Pike's Peak, the ocean and outside air in Boston, the highest result was 20·938 % oxygen and the lowest 20·927 %, with a carbon dioxide content of 0·031 %. One may conclude therefore that the closer the analyses of outside air approach 20·93 % for oxygen and 0·03 % for carbon dioxide the more reliable is the apparatus.

---

[1] Carnegie Institution Reports, No. 116.

In a large number of controls of the apparatus, the results were uniformly 20·92 % for oxygen and 0·03 % for carbon dioxide. To reach this degree of accuracy in the oxygen determination, great care must be taken in the preparation of the alkaline pyrogallate solution. The composition of the solution best adapted for the purpose is given in Benedict's monograph p. 80. We find it important to control the strength of the potassium hydroxide solution by a direct determination of its specific gravity—by weighing. No series of analyses were made until we had assured ourselves that the apparatus gave the correct CO$_2$ and oxygen content with outside air.

The general principle of the procedure was essentially that of Zuntz.

The air was delivered to the animal from a Hans Meyer respiration pump made by Rinck in Marburg, the air was taken from outside the laboratory and passed over water to saturate it partially with water vapour. The air passed to a tracheotomy tube and was delivered from the animal by means of a Y tube to a meter[1]. In the system on each side of the animal were by-passes, which allowed samples to be taken both of the incoming and of the expired air. As our object was to examine the metabolism of the animal at short intervals, sampling was done at intervals of thirty minutes to one hour.

The meter was read, taking the time with a stop-watch which was required for twenty litres of air to pass through the meter. The barometer and temperature of the air at the meter were noted. The meter readings were taken both before and after sampling and as a rule agreed well. Any differences were averaged. The sampling was done by filling the sampling tubes with dry mercury, attaching the upper end to the by-pass tube and after filling the by-pass tube with the air to be sampled allowing the mercury to run out into a vessel. No device was used, like that employed by Zuntz and by Grafe, to secure a uniform rate of flow. The mercury was run out so that between five and ten minutes were required to fill the sampling tubes, which had a volume of about 400 c.c.

---

[1] The meter was made by Alexander Wright & Co., and one revolution of the large hand indicated 2500 c.c. The dial was graduated to 10 c.c.

No attempt was made to obtain total readings on the meter. Our object was to ascertain the gaseous exchange at a given moment, and to compare it with what followed and what had preceded.

In many experiments when the voltage of the main current did not fluctuate, the readings throughout the day were constant to 2 °/₀. In other cases variations as high as 5 °/₀ were noted. As these variations did not occur during the time of sampling, the fluctuation did not introduce any error into the results.

Method of experiment. Dogs were used which had received no food for 17 to 20 hours. Under deep ether anæsthesia tracheotomy was performed and both carotids tied. The decerebration was then carried out by Langley's method. Into the distal end of the left carotid a cannula was tied, and through this a suspension of arrowroot starch, made by suspending five grams of starch in 50 c.c. of saline, was injected slowly. The injection was continued until the voluntary respiratory movements ceased, and reflexes were abolished from the eye of the opposite side. Usually 15 c.c. of the starch suspension were sufficient. The pump was then connected with the animal and artificial respiration applied. The femoral vein was exposed and a cannula inserted for obtaining venous blood for analysis. It would have been technically more satisfactory to obtain samples from the femoral artery, but as our object was to ascertain the composition of the blood after leaving the tissues we chose the vein. For this reason the number of blood samples is not as great as we could have wished, and in some instances thrombi interrupted the withdrawal of blood before the experiment was complete.

A catheter was introduced into the bladder which was washed out before the experiment. A thermometer was placed deep in the rectum. If the substance to be examined was to be introduced *per os* a stomach tube was put in before the animal was placed in a thermostat. When the substance was introduced intravenously a cannula was tied in the external jugular vein. In all but the earlier experiments an arterial cannula was tied in the right carotid and blood-pressure readings taken from time to time. When the operative procedures were finished the animal was placed in a water jacketed thermostat kept at about 28–29° C. This thermostat was probably of the type used by Tangl in his experiments. The top was closed by a number of glass doors, and the lids could be opened. The bottom and sides were water jacketed. It allowed any manual operations on the animal connected with the withdrawal of blood, urine, or the ascertainment of the blood-pressure to be done without opening the entire thermostat.

The starch method of decerebrating, as used by us, has certain disadvantages which are to some extent compensated for. By injecting starch until the respiratory movements ceased, we did away with the spontaneous movements of respiration which are seen in the animal when the fore brain is properly removed without interfering with the lower centres. The decerebrate rigidity which is experienced in the latter animals is transient in the starch animal and in the greater part of the experiments the general muscular tone was relaxed. Sometimes towards the end of a long experiment there was transitory spasm of the lower extremities. This was always noted and no readings are recorded when this occurred. These twitchings were invariably accompanied by a considerable rise in the total metabolism. The disadvantages which the amount of damage to the hind brain causes in the low blood-pressure are similar to what one obtains with compression in ordinary pithing with extravasation of blood into the cranium. In many cases no secretion of urine took place, so that we are unable to estimate the nitrogen metabolism during the experiment. This gap in our experiments we were unable to overcome. But as Loewy remarks, the nitrogen metabolism plays so small a part in the total metabolism that the inability to estimate it does not seriously affect the general results.

We have made experiments on decerebration in dogs, completely removing the brain with pressure on the vertebral arteries, after placing a stout ligature around the vertebral column in the hope of compressing the arteries between the atlas and axis. Even under these conditions when the dog is not suspended the amount of bleeding is very considerable. In this respect this animal is different from the cat.

The great advantage of the starch method from our point of view is of course its complete bloodlessness, although according to Baur[1] and Finckler[2] a considerable loss of blood is unaccompanied by a change in the total metabolism.

The recent work of Lusk[3] on the specific dynamic action of protein tends to show that certain of the cleavage products of protein are more potent in stimulating metabolism than are others, and it would seem that this function resides especially in the simplest of the amino acids, viz. glycocoll, while some fractions, notably glutaminic acid, exercise no stimulus at all.

---

[1] *Ztschr. f. Biol.* VIII. p. 567. 1872.

[2] *Pflüger's Arch.* X. p. 368. 1875.

[3] *Arch. Internat. Med.* XII. p. 485. 1913.

In an experiment which he regards as definitely settling the question Lusk fed a phlorhidinized animal with glycocoll, and determined the amount of sugar formed. Seventy per cent. of the sugar expected from glycocoll was obtained. The total metabolism increased 21 °/₀ over the usual value. Sugar alone administered did not increase the metabolism. Hence it appears that in glycocoll itself the stimulus is manifested, and this stimulus does not come through its own combustion. Benedict believes that the action is due to the organic acids, but Lusk found that ethyl glycollate gave no increase, while ethyl lactate did.

These very important considerations may be attacked from another side. If the effect of amino acids on metabolism is more or less confined to individual members of the class, and is not in relation to their catabolism, then the introduction of small amounts directly into the blood stream should be followed by quickly ensuing effects. We should also gain information as to the place of action by analyses of the blood, correlating the changes in composition of that fluid with changes in the total metabolism. It was from this point of view that the following experiments were undertaken.

It is naturally impossible to determine the total respiratory exchange on the intact animal after the intravenous injection of a substance, and of the two methods already discussed, the decerebrate animal appeared to us to present manifest advantages. The disadvantages we have encountered in this work have been already mentioned. Many of our animals were anuric, and in this respect the animals cannot be considered normal, for the waste products of protein metabolism were not removed from the blood and tissues.

Owing to the method elaborated by American investigators we are now in a position to determine to what extent the composition of the blood undergoes a change through the injection of nitrogen containing substances, and it is with the help of these methods that we may correlate the composition of the blood and the total metabolism.

Control experiments. In order to ascertain to what extent an animal subjected to the operations involved in the experiments would maintain an equable metabolism during the time of observation, controls were made in which no substances were administered.

Exp. I lasted six hours and twenty minutes from the time of taking the first sample of air and gives the changes which may take place. As will be seen, the amount of gaseous exchange is not constant. There is a progressive fall in the amounts of carbon dioxide excreted and the oxygen absorbed with practically constant rectal temperature. At the end of the fifth hour the rectal temperature rose suddenly, although the environmental temperature was the same as before and with the sudden rise in temperature the total metabolism rises over forty per cent. to fall at the end of the experiment when the rectal temperature had reached a practically fatal limit of 42·2°. The respiratory quotient for the animal remained exceedingly constant during the whole experiment with the exception of the fifth period, which falls out of the series, and for which no explanation can be given. The air was repeatedly analysed and gave constant results.

Unfortunately, as the amount of urine excreted in these experiments is no criterion of the extent of the protein metabolism, one is unable to make out the non-protein respiratory quotient.

The influence of glucose on the total metabolism.

Two experiments are offered, both of which involve the administration of glucose by the mouth. In the first experiment there was a distinct effect as shown by an increase in the total metabolism and by a sharp rise in the respiratory quotient. One fact must be pointed out which is seen in other experiments. In many cases the first respiratory quotient is high and seems to bear no relation to the state of nutrition of the animal. In this case the first respiratory quotient is 0·91 and sinks on the second and third observations to a constant level of 0·86. We are inclined to believe that this high preliminary value is associated with the anæsthetic used prior to decerebration, this despite the fact that the combustion quotient for ether is below 0·7.

Excluding the first period, the average for the second two observations is 82·3 c.c. of CO_2 per minute and the oxygen absorbed 95·3 c.c. per minute; with an average respiratory quotient of 0·86. Forty-five minutes after the ingestion of 50 grams of glucose in 150 c.c. of water, the carbon dioxide excretion has risen to 87·2 c.c. while the oxygen absorption has actually fallen with a consequent rise in the respiratory quotient to 0·94. In the second observation two hours after administration, the metabolism is practically of the same level, the respiratory quotient having risen to 0·98. For four hours the metabolism is maintained at this level, when a fall in the oxygen intake and carbon dioxide output occurs, indicating a lowering in the total metabolism. Up to the end of the experiment the respiratory quotient remains above 0·95. With failing total metabolism this decerebrate animal quantitatively makes use of the same relative amounts of fat and carbohydrate as at higher levels.

The second experiment is less distinctive but is of value as showing an animal whose initial metabolism was characterized by a very low respiratory quotient showing almost exclusively a combustion of fat. The effect of 14 grams of glucose *per os* was in this case transitory. The total metabolism of the animal was failing, for it lived but four hours after the administration of glucose.

The effect of the small amount of glucose was, however, similar to the preceding experiment. There was merely a maintenance of the oxygen absorption with increase in the carbon dioxide output with a rise in the respiratory quotient from 0·69–0·80.

The influence of protein on total metabolism.

The need for investigating the influence of protein on the total metabolism is not great, for our information on this subject is fairly complete. It was, however, necessary to perform some experiments of this kind in order to ascertain if the decerebrate animal reacts to protein feeding as does the intact one, and if the change in metabolism was synchronous with a change in the blood. Two experiments are reported which are not decisive for the reason that the animals were in a condition in which from the start the total metabolism was progressively sinking in intensity. In one case it was impossible to collect blood from the femoral vein, and in this instance no effect on the metabolism beyond a slight rise in the respiratory quotient is to be made out.

In the second there was undoubted resorption of protein as shown by the change in the composition of the blood. The total non-protein nitrogen rose from 33 milligrams per 100 c.c. to 39 after the administration of plasmon. The resorption was accompanied by a rise in the total metabolism which continued for nearly three hours. From the fourth period onwards there was a marked rise in the respiratory quotient, showing that the increase in metabolism was mainly at the expense of carbohydrate. When it is considered that the total metabolism throughout the experiment was decreasing, the rise due to the injection of protein becomes more significant. This can be shown by plotting the CO_2 output against the time and comparing the curve for the fore period with that for the after period. In this way, in spite of our inconstant total metabolism, one can obtain an idea of the effect of substances superimposed upon it. It seems therefore that the decerebrate preparation, with its shortcomings, reacts to protein almost as sharply as does the intact animal. We have not attempted to make any calculation of this effect but it certainly is large.

The influence of amino acids on the total metabolism.

The main point of this investigation was to ascertain whether the effects observed by Lusk[1] on the intact animal were to be obtained with the decerebrate preparation, and for this purpose we have made a number of experiments. We have confined ourselves to glycocoll, the amino acid which in Lusk's experiments gave the largest catalytic effect, and we have administered the acid either in small quantities intravenously or in larger quantities *per os*.

Three experiments with each mode of administration are given. In two of the experiments where the amino acid was given *per os*, the fore period indicates a falling metabolism and as a consequence no effect is observed. In these two experiments, however, there are some points of interest. In Exp. VII the second after period indicates an increase in metabolism coincidently with an increase in the non-protein nitrogen increase in the blood. There is also a decided increase in the respiratory quotient from 0·81 to 0·88.

The second experiment is difficult of interpretation. In the fore period the metabolism fell decidedly and a continuous fall is experienced in the first part of the after period. Just before the eighth period the rate of ventilation was changed so that the animal received 7900 c.c. of air per minute as against a previous 5400 c.c. The amount of oxygen absorbed and carbon dioxide excreted rose markedly, and with this there was a temporary rise in the respiratory quotient, indicating a sweeping out of carbon dioxide from the organism. It is noteworthy in this experiment that the analytical results give no indication of the resorption of an amino acid into the blood. The amount of non-protein nitrogen is perfectly constant.

The third experiment does show, however, that glycocoll very markedly affects the metabolism and this is associated with a rise in the non-protein nitrogen of the blood. Although blood-pressure and pulse were lower in the period during which the amino acid was having its effect the carbon dioxide output was distinctly higher. With this rise, the respiratory quotient rises to that associated with the combustion of protein after having been at a fasting level, and remains at this level throughout the experiment.

The total number of calories of the ingested glycocoll was 50·5. The weight of the dog 9 kilos. Estimating the needs of this animal at 60 cal. per kilo. per day the amount per hour would be $\frac{60 \times 9}{24} = 22{\cdot}5$ cal.

---

[1] *Journ. Biol. Chem.* XIII. p. 162. 1912.

Complete utilization of the glycocoll would therefore suffice for only two hours. The resorption was undoubtedly incomplete. Hence the glycocoll was responsible for only a very small part of the total metabolism of these periods, for we know that part of the amino acid is always excreted in the urine, and there is much evidence to show that the complete degradation of the amino acid takes a considerably longer time than that elapsing in these experiments. Despite these facts, the stimulating action as shown by the respiratory quotient lasted throughout the experiment, an elapsed time of five hours. It may be argued that the stimulation is only shown in the first two observations. If the curves of the fore period and after period are plotted out, it will be found that the metabolism of the after period is on a considerably higher plane than that of the fore period.

The following experiments were made to settle a question undetermined by Lusk's experiments, viz. do amino acids injected directly into the blood stream produce the same effect as when reaching the tissues by absorption from the alimentary tract? It might be expected that in the former case the effect would be more potent. The present experiments support this view although the infusion of the solutions was made with considerable slowness. The idea was to bring the amino acids slowly to the tissues without the interposition of the liver. In the first two experiments 10 grams of glycocoll were administered through the external jugular vein in 200 c.c. and 100 c.c. of saline respectively. In the third experiment 5 grams were infused in 120 c.c. of saline. In every instance a rise in the total metabolism was experienced. The rise was of such a nature as to preclude the possibility of an adventitious effect. Even in the experiment where only 5 grams of glycocoll were given the effect was well marked. In every case also there was a rise in the respiratory quotient, in some instances to the protein value; in others considerably beyond it. In the first experiment 5 grams of glycocoll dissolved in 120 c.c. of saline were injected into the external jugular vein, the time of injection occupying sixty-five minutes. Even with a slightly falling metabolism the rise, as the result of the injection, is most marked and it occurred both in carbon dioxide output and oxygen absorption. With this rise is associated a rise in the respiratory quotient from 0·73 to 0·79. The rise calculated from the carbon dioxide output is nearly 10 °/₀, and considering the interval between Obs. II and Obs. III, and the fact that the metabolism was falling, it is probably very considerably more. Unfortunately owing to a thrombus we were unable to collect the blood from the animal for analysis.

In the second experiment ten grams of glycocoll were infused into a dog weighing 10·3 kilos. The time of injection was 75 minutes. In this experiment we have a very decided effect on the total metabolism. The carbon dioxide output of the first after period taken ten minutes after the end of the injection shows an increase of 25 °/₀ over the last period.

In this experiment also there is a rise in the respiratory quotient which lasts for two hours, after which it falls to a practically fasting level.

The blood analyses do not disclose an increase in the total non-protein nitrogen, although in the urea values we have an indication of increased urea formation in the tissues. The urea nitrogen after being practically constant in the fore period rises nearly 40 °/₀ half an hour after the end of the injection.

In the third experiment a small dog weighing 6·25 kilos. received ten grams of glycocoll intravenously in thirty minutes. In this case the intention was to flood the organism with a large quantity of glycocoll. That this was accomplished is made evident by the analyses of the blood. The total non-protein nitrogen which was 34–32 mgrms. per 100 c.c. of blood during the fore period, rose during the after period to 55 mgrms. per 100 c.c. of blood, and with this rise there was a marked rise in the total metabolism. Both carbon dioxide excretion and oxygen absorption rose; the former to 125 °/₀ of the fore period excretion—the oxygen absorption increased 15 °/₀. The rise was only temporary for the animal was rapidly failing. An analysis of the expired air an hour and a half after the fifth sample contained only 0·02 °/₀ of expired carbon dioxide.

The experiment, although of short duration, showed the marked effect of glycocoll in stimulating the metabolism of a failing animal.

SUMMARY.

1. The decerebrate preparation responds to carbohydrates and proteins as does the intact animal.

2. The rise in total metabolism following the administration of protein is accompanied by a rise in the non-protein nitrogen in the blood.

3. The decerebrate animal reacts to small amounts of the amino acid tried, viz. glycocoll, with an immediate and very considerable rise in

total metabolism. The experiments of Lusk who gave this amino acid by mouth are confirmed and are further substantiated by the fact that the administration of this acid directly into the blood stream is even more prompt and effective in increasing metabolism than when the amino acid passes into the general circulation with the interposition of the liver.

We are indebted to the Government Grant Committee of the Royal Society for a grant to one of us (W.) towards the expenses of this research.

Exp. I. Dog, weight 9 kilos. Control.

	Time	Temperature		C.c. per min.		R. Q.	Pulse	B.-P. in mm. Hg.	Time	Obs.	Per 100 c.c. blood mgrms.	
		Cage	Animal	CO_2	O_2						Non-protein N	Urea N
I	11.56	28·2	38·6	74·2	88·0	0·84	156	—	11.7	I	34	12·5
II	12.30	28·4	38·3	61·9	75·5	0·82	144	—	12.10	II	29	15·0
III	1.40	28·9	38·3	53·2	64·8	0·82	180	—	1.30	III	25	15·0
IV	2.37	28·3	38·3	61·5[a]	75·9[a]	0·81	144	—	2.30	IV	25	13·7
V	3.10	28·3	38·3	59·1(1)	65·0	0·91	—	—	3.40	V	29	18·2
VI	4.40	28·4	39·7	76·2(2)	94·0	0·81	126	—	5.12	VI	43	27·8
VII	5.25	28·4	40·1	82·0(3)	100·0	0·82	146	—	6.23	VII	48	25·0
VIII	6.20	—	42·2	76·3	84·4	0·90	—	—				

(1) Ventilation 4450 c.c. per min.
(2) Ventilation 5570 c.c. per min.
(3) Ventilation 5000 cc. per min.
[a] Slight tremor.

Exp. II. Dog, weight 11·5 kilos. 50 grams of glucose in 150 c.c. of water *per os* at 12.42.

I	10.52	29·2	38·3	90·5	98·8	·91	160	—	10.28	I	31	12
II	11.20	30·4	38·5	88·2	96·2	·86	162	—	11.30	II	24	—
III	12.11	32·1	39·0	81·4	94·4	·86	160	—	12.38	III	31	—
IV	1.32	29·2	39·8	87·2	92·6	·94	148	—	1.54	IV	18	12
V	2.40	28·0	40·0	89·7	91·4	·98	146	—	2.52	V	28	—
VI	3.17	27·0	40·2	98·5	97·4	·96	142	—	3.52	VI	21	—
VII	4.00	26·5	40·2	77·6	79·2	·98	140	—	4.55	VII	36	—
VIII	4.45	26·3	40·0	81·1	84·3	·97	140	—				
IX	5.15	26·7	38·8	80·6	84·2	·96	136	—				

Exp. III. Dog, weight 6·25 kilos. 14 grams of glucose in 300 c.c. of water at 12.0.

I	11.7	32·0	38·4	48·9	68·2	0·71	176	—	10.45	I	26·0	11·1
II	11.36	—	38·2	45·4	66·1	0·69	192	—	11.58	II	26·0	10·9
III	12.27	29·0	37·8	52·7	65·8	0·80	208	—	1.18	III	24·0	10·7
IV	1.43	—	37·3	31·9	42·3	0·75	—	—	2.15	IV	28·0	9·8
V	2.32	28·5	37·2	27·9	38·6	0·72	—	—	3.34	V	32·0	9·5
VI	3.7	30·5	36·9	25·1	36·2	0·69	197	—	4.00	VI	23·0	10·9
VII	3.44	30·0	36·2	26·3	35·9	0·73	—	—				

Exp. IV. Dog, weight 7·25 kilos. 20 grams of plasmon in 200 c.c. of water at 11.40.

I	10.40	—	37·5	42·3	49·0	0·86	164	116
II	11.13	—	36·9	38·3	44·3	0·86	—	—
III	11.45	—	26·3	36·2	40·9	0·88	128	88
IV	12.30	—	—	34·5	37·9	0·91	—	—
V	1.20	28·7	35·9	31·8	34·5	0·92	118	74
VI	2.31	—	35·2	30·9	37·5	0·82	126	74
VII	3.34	—	35·2	29·4	35·3	0·83	—	—
VIII	4.7	29·6	35·1	28·1	33·4	0·84	—	74
IX	4.37	—	—	30·2	35·7	0·84	—	—

Exp. V. Dog, weight 7·0 kilos. 20 grams of plasmon in 150 c.c. of water at 12.0.

I	11.7	27·8	36·4	42·8	55·9	0·77	106	106	10.40	I	33	10·0
II	11.41	—	35·7	40·5	53·4	0·76	100	100				
III	12.16	30·7	35·7	48·8	62·6	0·78	—	98	12.25	II	38	15·6
IV	1.31	29·7	36·0	42·8	49·0	0·87	83	—	1.25	III	39	20·8
V	2.9	28·5	36·0	40·0	43·1	0·93	78	—	2.25	IV	33	15·4
VI	2.53	31·7	35·8	41·2	47·3	0·87	78	66	3.8	V	—	27·8
VII	3.41	32·0	35·8	32·7	36·3	0·90	86	—	4.7	VI	—	28·6
VIII	4.24	—	35·7	19·7	21·6	0·91	88	48				

Exp. VI. Dog, weight 6·0 kilos. 25 grams of plasmon in 200 c.c. of water at 12.40.

I	11.5	28·9	38·3	38·5	46·0	0·84	194	—	10.15	I	22	11·7
II	11.37	29·5	37·5	34·8	44·0	0·79	172	—	11.55	II	22	13·3
III	12.25	29·0	37·0	32·5	40·4	0·80	154	—				
IV	1.51	28·7	36·8	25·6	36·7	0·70	154	—	2.0	III	32	16·7
V	2.30	28·4	36·3	21·0	29·5	0·72	152	—	3.7	IV	33	17·0
VI	3.20	28·0	—	20·2	22·2	0·91	134	—				

Exp. VII. Dog. 15 grams glycocoll in 150 c.c. of water at 12.30.

I	11.26	29·3	36·4	41·8	51·8	0·81	122	94	11.0	I	22·0	10·4
II	12.4	30·4	36·1	37·2	45·9	0·81	112	—				
III	12.53	30·6	36·0	32·4	40·2	0·81	106	88	1.47	II	31·0	16·1
IV	1.30	—	—	33·8	41·2	0·82	104	—	2.50	III	41·0	20·0
V	2.9	30·6	35·9	30·8	38·0	0·81	100	90	3.45	IV	51·0	16·7
VI	2.37	—	—	29·8	35·1	0·85	—	—	4.37	V	47·0	18·5
VII	3.17	30·9	35·6	24·7	29·3	0·84	104	62				
VIII	4·0	28·0	35·5	20·1	23·0	0·88	—	—				
IX	4.28	28·0	35·3	22·2	26·2	0·85	—	62				

Exp. VIII. Dog, weight 12·3 kilos. 10 grams of glycocoll in 100 c.c. of water *per os* at 11.58.

I	10.30	28·5	37·5	84·8	98·5	0·86	190	—	10.16	I	29·5	16·7
II	11.0	29·3	37·3	71·7	94·1	0·76	180	—	11.30	II	29·0	15·6
III	11.43	29·4	37·3	63·3	85·8	0·74	170	—				
IV	12.32	29·3	37·3	60·2	75·3	0·80	156	—	12.45	III	29·0	16·7
V	1.6	29·5	37·2	56·6	71·6	0·79	144	—	2.15	IV	29·4	—
VI	1.55	29·5	37·2	55·9	72·0	0·78	138	—	3.16	V	29·5	20·8
VII	2.41	29·3	37·0	47·7	63·2	0·75	132	—	3.45	VI	29·0	21·3
VIII	3.30	28·7	37·0*	49·2	68·0	0·73	130	—	4.45	VII	29·0	23·8
IX	3.51	29·0	37·0*	71·5	83·2	0·86	130	—				
X	4.41	29·4	37·3*	71·0	94·8	0·75	?	—				

* Rate changed.

Exp. IX. Dog, weight 9·2 kilos. 15 grams of glycocoll in 150 c.c. of water *per os* at 11.20.

I	10.30	28·5	38·3	84·0	104·4	0·80	172	124	10.05	I	31	12·3
II	11.7	28·5	37·9	70·3	98·3	0·71	164	—				
III	11.33	30·0	37·8	—	—	—	158	104	11.52	II	36	12·5
IV	12·7	30·4	37·7	77·0	92·1	0·80	148	—	12.48	III	36	14·5
V	12.28	29·8	37·8	73·0	90·0	0·81	136	96				
VI	1.6	28·6	37·8	66·4	83·2	0·80	—	—				
VII	1.40	—	—	59·2	75·2	0·77	126	—				
VIII	2.16	—	—	57·3	72·8	0·78	—	—				
IX	3.3	30·6	36·6	51·8	66·4	0·78	108	98				
X	3.45	30·2	36·5	56·0	64·8	0·79	104	—				
XI	4.15	29·2	36·5	49·9	62·1	0·80	104	—				

Exp. X. Dog, weight 8·2 kilos. 5 grams of glycocoll in 120 c.c. of saline intravenously from 1.5 to 2.11.

		Temperature		C.c. per min.							Per 100 c.c. blood mgrms.	
	Time	Cage	Animal	CO_2	O_2	R. Q.	Pulse	B.-P. in mm. Hg.	Time	Obs.	Non-protein N	Urea N
I	11.40	27·8	37·5	34·2	45·7	0·75	—	—				
II	12.31	26·7	37·0	33·5	45·5	0·73	—	—				
III	2.8	29·4	36·2	36·7	48·0	0·76	—	—				
IV	3.0	29·4	36·5	35·3	44·8	0·79	—	—				
V	4.2	29·9	36·7	32·9	43·1	0·76	—	—				
VI	4.50	31·0	36·8	34·2	45·1	0·75	—	—				

Exp. XI. Dog, weight 10·3 kilos. 10 grams of glycocoll in 200 c.c. of saline intravenously 1.0 to 2.15.

I	10.50	26·0	37·5	69·0	84·4	0·82	180	—	10.28	I	29	12
II	11.31	30·6	37·8	61·0	79·6	0·76	160	—	11.46	II	34	10
III	12.11	29·9	38·1	57·1	75·4	0·75	156	—	12.57	III	23	13
IV	12.43	29·6	38·3	53·2	71·2	0·74	154	—				
V	2.10	27·9	38·6	67·1	83·5	0·80	156	—	2.44	IV	25	17
VI	3.0	28·5	39·2	66·2	78·6	0·83	156	—				
VII	3.54	27·9	39·6	63·0	86·8	0·73	158	—				
VIII	4.35	27·7	39·8	66·1	92·0	0·72	160					

Exp. XII. Dog, weight 6·25 kilos. 10 grams of glycocoll in 150 c.c. saline at 12.5—12.35.

I	10.45	28·5	39·3	36·2	45·9	0·74	166	—	10.25	I	34·0	13·5
II	11.23	31·0	39·3	35·9	41·4	0·88	160	—	11.34	II	32·0	16·8
III	11.44	31·1	39·4	32·0	37·0	0·86	—	—	11.55	III	32·0	17·2
									12.43	IV	55·0	25·6
IV	12.54	30·0	38·6	39·9	43·3	0·90	142	—	1.25	V	55·0	25·0
V	1.34	29·6	38·5	25·3	26·4	0·93	—	—	2.30	VI	52·0	9·8

THE SUPRARENAL BODIES AND DIURESIS.

BY DOUGLAS COW (*Beit Memorial Research Fellow*).

(*From the Pharmacological Laboratory, Cambridge.*)

WHILST carrying out perfusion experiments on the kidneys of cats I was struck by the different effects produced by perfusing (*a*) directly through the renal arteries and (*b*) through the aorta.

The kidneys were perfused *in situ*, the intestines only (together with the spleen) having been removed between ligatures.

(*a*) In the first series of experiments the afferent cannulæ were inserted in the renal arteries: the perfusion fluid escaped from cannulæ inserted in the inferior vena cava, or in the renal veins if it was desired to record the circulation through the two kidneys separately: whilst the urine was collected by means of the bladder-cannula which I have described elsewhere[1].

(*b*) In the second series of experiments the afferent cannula was tied into the thoracic aorta immediately above the diaphragm: all vessels that could be seen coming from the aorta were ligatured except the renal arteries, and the aorta was ligatured below the renal arteries. The perfusion fluid issuing from the kidneys and the urine was collected as in the first series.

The perfusions were carried out at a constant pressure of 80–90 mm. Hg. The perfusion fluid was kept at a constant temperature by means of a water-bath, the temperature of the fluid as it entered the vessels being about 36° C. The urine was collected every ten minutes and accurately weighed in a tared flask.

During the first 30–45 minutes or more of each experiment oxygenated Ringer's solution was perfused. The experiments in which the perfusion fluid flowed directly into the renal arteries showed invariably a progressive rise in the amount of urine, usually (but not always) accompanied by a progressive increase in the rate of flow of the perfusion fluid (Fig. 1). Those experiments in which the perfusion was made into the aorta, on the other hand, showed a progressive fall in the amount of urine, accompanied by a progressive fall in the rate of flow of the perfusion fluid (Fig. 2). Sometimes when the perfusion was continued for a longer period (4–5 hours) the increase or diminution in the flow of urine was not constant, though it always was so for at least two hours after the commencement of the perfusion. This agrees in the main with Sollman's results in perfusing the isolated kidney[1].

---

[1] Cow. This *Journal*, XLVIII. p. 1. 1914.

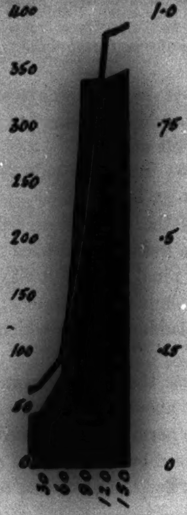


Fig. 1.

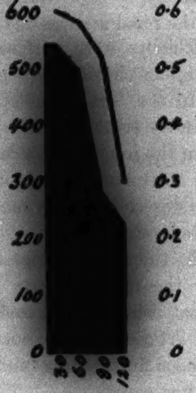


Fig. 2.

Fig. 1. Rate of flow of perfusion fluid and of urine. Kidneys perfused with Ringer's solution directly into the renal arteries. The single line = perfusion fluid. The shaded curve = urine flow. Ordinate = c.c. of perfusion fluid and c.c. of urine. Abscissa = time in minutes.

Fig. 2. Rate of flow of perfusion fluid and of urine. Kidneys perfused with Ringer's solution through the aorta. The single line = perfusion fluid. The shaded curve = urine flow. Ordinate = c.c. of perfusion fluid and c.c. of urine. Abscissa = time in minutes.

After excluding all possible sources of difference in the conditions of the experiments, the only feasible explanation appeared to be that in those experiments in which the perfusions were made into the aorta there was a circulation through the suprarenal bodies, though under the conditions in which the perfusions were carried out it was not easy to understand in what way fluid perfused through the suprarenal bodies could reach the fluid flowing through the kidney vessels.

---

[1] Sollman. *Amer. Journ. of Physiol.* XIII. p. 241. 1905.

An experiment was then performed as follows:—a cat, anæsthetized first with A.C.E. mixture and then with urethane, was eviscerated (stomach, intestines and spleen): a cannula connected with the perfusion fluid reservoir was tied into the aorta just above the diaphragm: cannulæ were tied into the two renal veins: the aorta was ligatured below the renal arteries and all branches of the aorta that could be seen between the afferent cannula and the renal arteries were ligatured. The ureters were catheterized with capillary glass tubes introduced by means of horsehair probes. The kidneys were then perfused with Ringer's

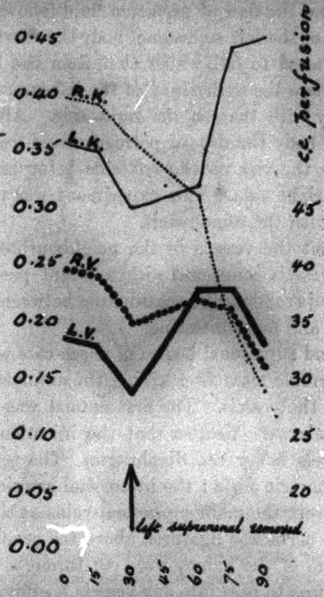


Fig. 3. Rate of flow from renal veins and from ureters. Kidneys perfused with Ringer's solution through the aorta. Ordinate=c.c. of perfusion fluid and c.c. of urine. Abscissa=time in minutes. ·········=flow from right ureter (*R.K.*). ——=flow from left ureter (*L.K.*). ●●●●●=flow from right renal vein (*R.V.*) ▬▬▬=flow from left renal vein (*L.V.*) At the point indicated the left suprarenal was ligatured off and removed.

solution at constant temperature (about 36° C.) and pressure (80 mm. Hg.). The perfusion fluid and urine were collected separately from each kidney and accurately measured every 15 minutes. After perfusing for 30 minutes the left suprarenal body was quickly ligatured off and extirpated. The result of this experiment is shown in graphic form in Fig. 3. During the first 30 minutes the flow of urine from the two kidneys and the flow of perfusion fluid followed parallel curves. After the removal of the left suprarenal body the flow of urine from the right kidney continued to fall, whilst that from the left kidney began to rise, whereas before the extirpation of the left suprarenal body it was falling in company with that on the right side. After the removal of the left suprarenal body the flow of perfusion fluid from the renal veins rose on both sides, the rise on the left side being much more marked than that on the right side. This experiment was repeated on three other cats always with the same result.

It is known that the vessels in the neighbourhood of the kidneys anastomose with vessels connected with adjacent parts, thus Tuffier[1] and Quenu and Lejars[2] describe anastomoses between the vessels of the kidney, suprarenal and large intestine.

The kidneys and suprarenal bodies of three cats were then injected with a gelatine-carmine mass after killing the animals with coal-gas, in order to dilate all the vessels. The first animal was injected through the aorta: no vessels were tied, so that the injection mass was free to pass into any vessels below the diaphragm. The second animal was injected into the thoracic aorta: the intercostal arteries and cœliac axis were tied, as also were the main suprarenal veins at their entrance into the vena cava: the aorta was ligatured above the renal arteries and the vessels in the hilum of the kidney were cut through. In this case the injection mass was free to pass into any vessels leading to the suprarenal bodies from either the aorta or the phrenic arteries: the injection mass could not reach the kidneys excepting through vessels entering them through the capsule. The third animal was injected into the renal artery direct, the cannula being pushed as far into the pelvis of the kidney as possible. A ligature was tied round all the tissues between the renal artery and the suprarenal body, in order to prevent the injection mass reaching the suprarenal body through any branches of the renal artery supplying the suprarenal body. The renal vein was cut and as soon as the injection mass issued therefrom it was tied.

---

[1] Tuffier. *Revue de Chirurgie*, p. 390. 1890.

[2] Quenu and Lejars. *Etude sur le systeme circulatoire*, Paris, 1894.

The injection was then continued. After injection the tissues were hardened *in situ* before removal for dissection.

The first injected specimen showed a fine rete of vessels formed from branches of the aorta leading to the suprarenal bodies. The main drainage of the suprarenal bodies was into the large veins which cross them, one on each side, immediately before joining the inferior vena cava. Behind these veins and embedded in the peri-renal fat was another fine rete of vessels coming from the suprarenal bodies. This rete joined up into a small vessel which supplied branches entering the kidney through the capsule around the convex border of the kidney. Accompanying some of these vessels entering the kidney were other

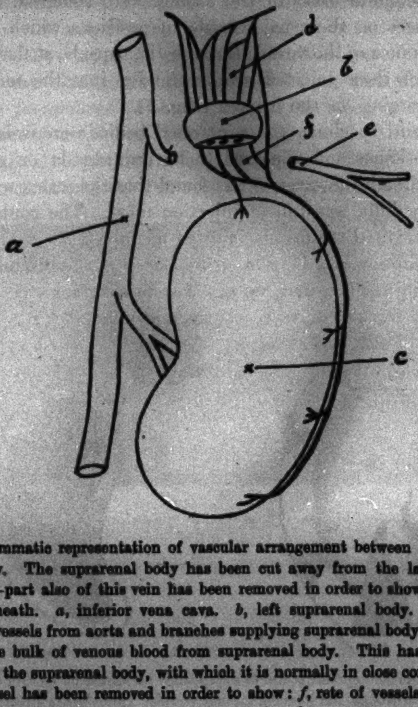


Fig. 4. Diagrammatic representation of vascular arrangement between suprarenal body and kidney. The suprarenal body has been cut away from the large vein which crosses it—part also of this vein has been removed in order to show the fine rete of vessels beneath. *a*, inferior vena cava. *b*, left suprarenal body. *c*, left kidney. *d*, rete of vessels from aorta and branches supplying suprarenal body. *e*, vein which receives the bulk of venous blood from suprarenal body. This has been dissected away from the suprarenal body, with which it is normally in close contact. Part too of this vessel has been removed in order to show: *f*, rete of vessels between suprarenal body and kidney. These vessels are for the most part embedded in the peri-renal fat and supply branches which dip beneath the capsule of the kidney.

vessels (obviously veins) which on leaving the kidney joined up into a vessel which emptied itself into the main suprarenal vein. The general arrangement of these vessels is shown in Fig. 4.

Similar vascular connections were found in the other two specimens. In the case of the second specimen the kidney was cut open longitudinally and it was found that the injection mass had entered the kidney and that cuneiform areas of the kidney were coloured. These areas had their bases on the renal capsule in positions which corresponded with the entrance of the vessels through the capsule, as described above. The apices of these injected areas extended into the medulla of the kidney, converging on the calyces (Fig. 5).

In the third specimen the suprarenal bodies were isolated from the surrounding tissues and appeared to be uninjected: on cutting them open longitudinally, however, it was found that the cortex was uninjected whilst the medulla was full of injection mass. The contrast between the pale cortex and the deeply injected medulla was extremely marked. With the exception of a portion of the cortex at the hilum, where there were many injected vessels, only a few minute injected vessels were seen crossing the cortex to reach the medulla (Fig. 6).

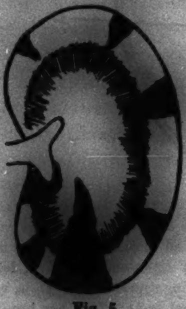


Fig. 5.

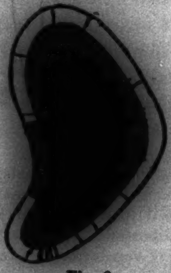


Fig. 6.

Fig. 5. Diagrammatic representation of appearance presented by section of kidney which was injected through the reno-suprarenal vessels. The darkly shaded parts indicate the parts of the kidney showing injection.

Fig. 6. Diagrammatic view of section through suprarenal body which was injected with gelatine-carmine mass through the reno-suprarenal vessels. The shaded parts indicate those parts injected.

It appeared, then, that there exists at any rate a possible channel through which the products of the suprarenal bodies can reach the kidneys directly and without either dilution or oxidation in the general circulation. In order to clinch the matter a cat under urethane was bled from (*a*) the carotid artery, (*b*) the femoral artery, (*c*) the renal vein and (*d*) the stripped off kidney capsule, whilst the sciatic nerve was being stimulated[1]. The main suprarenal vein in this animal had been ligatured close to its junction with the vena cava, in order that the products of the suprarenal bodies might not be poured out into the general circulation. These samples of blood were whipped and filtered through glass-wool and diluted with an equal volume of Tyrode solution. Portions were then injected intravenously into a cat under urethane, and though the blood from the stripped kidney capsule appeared to have a more pronounced pressor action on the blood-pressure than the other samples of blood the difference was too small to be relied on. The more sensitive method described by **Dale** was then tried. The uterus of a guinea-pig was removed and suspended in a bath of warm (36° C.) Tyrode solution, so that it pulled on a counterbalanced lever. On adding small quantities of the different samples of blood to the Tyrode bath it was found that, whereas the blood from the femoral and carotid arteries and from the renal vein produced no effect on the uterine contractions, the sample collected from the stripped off kidney capsule had a strong pressor action, identical indeed with that of adrenalin, as was shown afterwards on the same isolated organ. These results were confirmed by repetition of the experiment on other animals

Fig. 7. Pregnant uterus of guinea-pig in bath of Tyrode solution at 36° C. At 1, 1 c.c. of blood from the carotid artery; at 2, 1 c.c. of blood from the renal vein; and at 3, 1 c.c. of blood from the stripped kidney capsule of a cat were added to the solution. Upstroke = contraction.

---

[1] It has been shown by **Elliott** (This *Journ.* XLIV. 374. 1912) that stimulation of the sciatic nerve produces a reflex exhaustion of the adrenalin content of the suprarenal bodies.

and by repeated applications of the various samples of blood in each case (Fig. 7). No appreciable diminution in the pressor activity of the renal capsule blood was found even when it was collected from an animal in which the main suprarenal vein had not been tied.

It is obvious, then, that the products of the suprarenal body not only can but under certain conditions do actually enter the kidney directly without previous dilution or oxidation in the general circulation.

It has been shown by **Asher and Pearce**[1] that stimulation of the splanchnic nerve causes a diminution in the flow of urine, whilst, with the splanchnics cut through, stimulation of the vagus is followed by an increase in the amount of urine. These authors suggest, though this is denied by others, that the vagus and splanchnics are direct secretory nerves to the kidney.

Elliott[2] shows that fright or excitation of afferent nerves causes a reflex exhaustion of the adrenalin content of the suprarenal bodies, and that the efferent path of this reflex is by the splanchnics.

If it can be demonstrated, then, that adrenalin has the same action on the flow of urine as has stimulation of the splanchnics, there is at hand an obvious explanation of the results of **Asher and Pearce** without introducing the hypothesis that the kidney has a direct secretory innervation: namely that stimulation of the splanchnic nerves produces an outflow of adrenalin from the suprarenal bodies and that some at any rate of this adrenalin finds its way directly to the kidney, producing a diminution in the flow of urine.

That this is the case is shown by the following experiment: a cat anæsthetized with urethane was eviscerated (stomach, intestines and spleen). The right ureter was tied, the right suprarenal body was extirpated and the left suprarenal vein was ligatured close to the vena cava. Thus no suprarenal products could reach the general circulation except after passage through the left kidney. The urine from the left kidney was collected and a drop record taken. After leaving the animal to recover from any shock due to the operation the sciatic nerve was stimulated. Before the stimulation the urine was flowing at the rate of 4·5 drops per min.: during the stimulation the flow rose for a short period to six drops per min., then rapidly fell to about two drops per min. and remained at or about this level for a considerable time. Later, when the rate of flow had recovered an intravenous injection of adrenalin was given at a time when rather less than four drops of urine were being registered per min. The urine flow rose at once to 7·5 drops per min.: this rise was but momentary and was followed by a fall to about one drop per min., which rate was maintained for some time. These two curves are shown in Fig. 8. On repeating this experiment precisely similar results were obtained.

---

[1] **Asher and Pearce.** *Ztschr. f. Biol.* LXIII. p. 83. 1913.

[2] **Elliott.** *Loc. cit.*

A few experiments were then carried out with the idea of showing that possibly pilocarpine might produce an increased flow of urine when the suprarenal bodies were intact, and that it might be without this effect after extirpation of the suprarenals. Though seven experiments were performed the results were not conclusive; though on one occasion an injection of pilocarpine produced an increase in the flow of urine from about one drop in two minutes to more than four drops per minute, whilst the blood-pressure fell at the same time from about 65 mm. Hg. to about 20 mm.

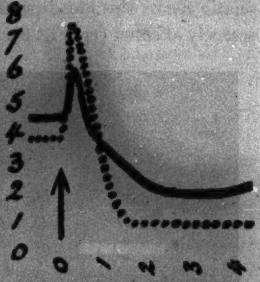


Fig. 8. Urine flow, ——— affected by stimulation of the sciatic nerve, and •••• affected by injection of adrenalin. The stimulation and injection were applied at the point indicated. Ordinate=urine in drops per minute. Abscissa=time in minutes.

Since the differences in the flow of urine dependent on the presence or absence of the suprarenals are at times out of all proportion to the differences produced in the amount of fluid flowing through the kidney vessels, it appears likely that the action of adrenalin on the kidney is not so much on the musculature of the renal vessels as on some part perhaps of the calyx or pelvis of the kidney. In this case the probable explanation of its action is that it causes some part of the pelvis of the kidney to contract, with a sudden consequent gush of urine into the bladder, and that after this initial expulsion of urine the flow of urine remains depressed as long as the contraction due to the adrenalin is maintained. This hypothesis is to a certain extent borne out by a phenomenon of common experience, viz. the effect of fright or acute nervous apprehension, in which states the bladder is frequently unable, or with difficulty able, to retain the urine suddenly poured into it. Similarly the polyuria occasionally met with in Addison's disease may perhaps be explained by the assumption that inadequacy of adrenalin brings about inadequate tonic control of the kidneys.

Two further experiments were performed in the following way: a cat anæsthetized first with A.C.E. mixture and then with urethane was eviscerated; the sciatic nerve was isolated and placed on electrodes; both ureters were catheterized with capillary tubes, and the urine was thus collected separately from the two kidneys, a drop record being taken of the flow from each: the blood-pressure was recorded from the carotid artery by a mercurial manometer in the usual way. Before the commencement of the experiment the flow of urine was increased by an intravenous injection of hypertonic saline solution. On stimulating the sciatic nerve the blood-pressure rose sharply and the flow of urine was scarcely affected (cp. Fig. 9). The left suprarenal vein was then clamped with small bulldog forceps at its point of entry into the inferior vena cava in order to prevent any of the efferent blood from the suprarenal body on that side from passing directly into the general circulation: and the urinary flow was quickened by intravenous injection of hypertonic salt solution. On stimulating the sciatic nerve once more the blood-pressure again rose sharply, the flow of urine from the right kidney as before was scarcely affected, whilst the flow of urine from the left kidney was abruptly shut off, and remained in abeyance so long as the sciatic nerve stimulation continued (shown by the band in the signal line Fig. 9) and for some time afterwards.

Fig. 9.

SUMMARY.

1. A direct vascular connection between the suprarenal bodies (medullary portion) and the kidneys is described.

2. Under certain conditions adrenalin in appreciable amount is poured directly into the kidneys from the suprarenal bodies, producing a diminution in the flow of urine.

3. The suprarenal bodies may be regarded as direct regulators of urinary activity.

THE TEMPERATURE COEFFICIENT OF THE REFRACTORY PERIOD IN NERVE. BY E. D. ADRIAN, *Fellow of Trinity College, Cambridge.*

(From the Physiological Laboratory, Cambridge.)

CONSIDERED apart from any other evidence, the effect of temperature on the rate of a process which takes place in living tissue, *e.g.* the rate of the heart beat, can give little or no information as to the nature of the process in question. It is true that the possession of a high temperature coefficient (between 2 and 3) is sometimes taken as a proof that the process is a chemical rather than a physical event[1]; however, the arguments which support this view are valid only when we make the assumption that a change of temperature has no effect on the composition of the system in which the process goes forward. In the case of protoplasm it is extremely unlikely that a change of temperature would have no effect at all on the state of aggregation of the colloids, the concentrations of different ions, etc. But if the composition of the system is affected by temperature in this way, then the total effect of a change of temperature on a process taking place in the system may be due as much to a change in the composition of the system as to the direct effect of temperature on the process. For instance, the rate of a chemical reaction might appear to have a very small temperature coefficient if the rate of the reaction varied with the concentration of free $H^{\cdot}$ ions, and the concentration of free $H^{\cdot}$ ions in the system varied inversely as the temperature. In the same way the absorption of light by the gaseous system $N_2O_4 \rightleftarrows 2NO_2$ has a high temperature coefficient because a change of temperature shifts the equilibrium point and so alters the concentration of the coloured component N_2O_4. However, the absorption of light is not a chemical process any more than the chemical reaction considered above is a physical process. Consequently the fact that the rate of conduction of the nervous impulse (*e.g.*) has a high temperature coefficient does not allow us to conclude that the conduction of the impulse is a chemical rather than a physical process unless we are prepared to maintain that a change of temperature has no effect at all on the composition of the very complex system of which the nerve is formed. Nor can we draw any conclusion of this kind from the fact that the rate of the process is a logarithmic or a linear function of the temperature, since the form of the relation may depend in the same way on the changing composition of the system rather than on the nature of the process.

---

[1] Although the precise meaning of this distinction is not very clear, particularly in the case of a colloidal system; and although many physical processes have very high temperature coefficients, *e.g.* the rate of evaporation of water between 90 and 100° C. at atmospheric pressure.

This point has been emphasised by **Lucas**[1] and by **Knowlton** and **Starling**[2], but the opposite view appears to be so widespread[3] that its discussion may be pardoned in a paper which deals entirely with the measurement of a temperature coefficient.

Such measurements are, however, not without value, since they give some information as to the relationship between different processes. For instance, **Lucas**[4] has been able to show that in muscle at any rate the propagated change resulting from an adequate stimulus is qualitatively different from the local change set up by an inadequate stimulus[5], because the after-effects of the two processes have different temperature coefficients. In the same way **Lucas**[6] has shown that the absolute refractory period does not always correspond with the ascending phase of the electric response (as **Tait** suggested). Another point of interest is the difference between the temperature coefficient of the refractory period and that of the rate of conduction.

From this it will be seen that the temperature coefficient of the refractory period is a quality which deserves accurate measurement. At present the only observations made with the express purpose of measuring it in striated muscle or medullated nerve are those of **Bazett**[7] on the sartorius of the frog. **Bazett** found that the curarised sartorius gave a value of about 3 for the quotient $\frac{\text{Refractory Period at } T^\circ}{\text{Refractory Period at } T\ 10^\circ}$ where T varied from 1° to 5° C. The refractory period of the curarised muscle was about double that of the uncurarised, and **Bazett** concluded that the short values given in the latter case were due to stimulation of the intramuscular nerves. The temperature coefficient of this short refractory period was about 3·6, and this value agreed with some of **Boycott's** experiments[8] on the gastrocnemius sciatic preparation stimulated indirectly. A very much lower value for nerve may be obtained from the measurements of Bramwell and Lucas[1] on the same preparation. These give a temperature coefficient between 1·6 and 1·8. It is true that they were not made with the express purpose of measuring the temperature coefficient, and consequently its value has to be deduced from a comparison of the refractory periods of different preparations at different temperatures and not from those of one and the same preparation at different temperatures. However this criticism applies in some measure to Bazett's determinations as well. Thus for nerve at any rate the value of the temperature coefficient is still very doubtful. An explanation of these divergent results is suggested by the fact that the interval which is actually measured in all these determinations is not the refractory period of the nerve, *i.e.* the period within which a second propagated disturbance cannot be set up in the nerve, but the least interval for muscular summation, *i.e.* the least interval at which two stimuli applied to the nerve will produce a summated contraction in the muscle. As Lucas and Adrian[2] have shown this interval depends both on the rate of recovery of the nerve and also on the decrement which the disturbance may have to undergo before it reaches the muscle. The relative importance of the second factor is extremely variable from one preparation to another, and, no doubt, from one temperature to another. Consequently it is not surprising that results based on the measurements of the least interval for muscular summation show considerable variations; in any case they cannot be taken to give a true value for the rate of recovery of the nerve at different temperatures. This objection does not apply to Bazett's measurements of the refractory period of the sartorius stimulated directly, since in this case it is probable that the decrement factor does not come into play.

---

[1] This *Journal*, XXXVII. p. 112. 1908.

[2] *Ibid.* XLIV. p. 217. 1912.

[3] **Snyder.** *Ztsch. f. allgm. Physiol.* XIV. p. 263. 1913. **Lillie.** *Amer. Journ. of Physiol.* XXXIV. p. 414. 1914.

[4] *Proc. Roy. Soc.* B, LXXXV. p. 501. 1912.

[5] **Bazett's** value for muscle is 300 %.

[6] **Lucas**, *loc. cit.* p. 510.

[7] This *Journal*, XXXVI. p. 426. 1908.

[8] *Ibid.* XXIV. p. 144. 1899.

The experiments to be described were made with a view to determining the effect of temperature on the rate of recovery of nerve, excluding as far as possible all the complications introduced by the decrement factor. The particular aspect of recovery dealt with was the recovery of excitability during the relative refractory period.

If we construct the "recovery curve" of a muscle nerve preparation, *i.e.* the curve relating the interval between two stimuli to the strength which the second must have if it is to produce a summated contraction, we find as a rule that the required strength of the second stimulus rises gradually, as the interval is reduced, until a certain limiting value has been reached; at this point the curve suddenly becomes parallel to the current-strength axis, and any further increase in strength fails to reduce the interval for summation by the smallest amount. This limiting value determines the least interval for muscular summation, and its value has been shown[1] to depend on the conductivity of the nerve between the seat of the second stimulus and the muscle. When the disturbance has very little decrement to undergo before reaching the muscle, the recovery curve often shows no discontinuity, but approaches a line parallel to the current-strength axis asymptotically[2]. In this case the least interval for muscular summation is determined by the asymptote to the curve. However, the presence or absence of a decrement in conduction affects only the least interval for summation; the gradual rise of the recovery curve at intervals greater than this has been shown to depend only on the condition of the tissue at the seat of stimulation and not at all on the state of the nerve between this point and the muscle. It is found that as long as the interval between the two stimuli is greater than the least interval for summation, any stimulus which is strong enough to overcome the local resistance to excitation is also strong enough to produce a summated contraction. It follows that the gradually rising part of the recovery curve (which relates the interval between the stimuli to the strength the second must have if it is to produce a summated contraction) is really an expression of the return of local excitability at the seat of the second stimulus. Consequently the measurement of this part of the curve will allow us to compare the rate of return of excitability in the nerve at different temperatures, and the accuracy of the result will not be affected in any way by alterations in the conductivity of the nerve, since these affect only the least interval for summation and not the form of recovery curve at longer intervals.

---

[1] This *Journal*, XLII. p. 508, Figs. 6 and 7. 1911. [2] *Ibid.* XLIV. p. 120. 1912.

Experimental.

The muscle nerve chamber used in these experiments was the same as that described in connection with some experiments on the rate of conduction in nerve[3]. The nerve could be completely immersed in Ringer's fluid at different temperatures, and stimulated at different points by means of slot electrodes at which the lines of current flow in the fluid were suddenly constricted round the nerve. Only one of these electrodes was used, as both stimuli were sent in at the same point on the nerve. The temperature of the nerve was controlled by

[1] Adrian. This *Journal*, XLVI. pp. 388 *et seq.* 1913.

[2] Both types of curve are shown in Fig. 3.

[3] This *Journal*, XLVIII. p. 58. 1914.

bathing it with mixtures of different proportions of hot and cold Ringer, or, for low temperatures, by using Ringer at 1° or 2° C. and allowing it to pass through a foot or more of rubber tubing, exposed to the air, before reaching the nerve. With this arrangement the temperature could be adjusted very easily by altering the rate of flow. The temperature was measured by a small Anschütz thermometer with its bulb immersed in the fluid at a point about 2 mm. distant from the stimulating electrode. The muscle was kept covered with Ringer's fluid at room temperature. No special precautions were taken to prevent alterations of two or three degrees in the temperature of the muscle brought about by cooling the nerve to very low temperatures; such precautions would have been quite superfluous, since the rate of return of excitability depends only on the conditions at the point stimulated and on nothing else.

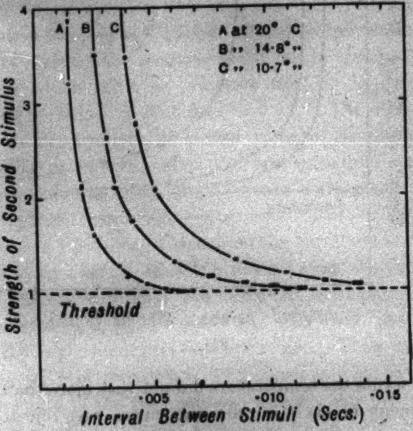


Fig. 1.

The usual method of determining the recovery curve has been described fully elsewhere[1]. The same method was used in the present case with the difference that the strength of the second stimulus was adjusted by inserting a variable resistance in the primary of the coil, instead of by altering the distance between the primary and secondary. This method allows one to dispense with the calibration curve, which is difficult to determine with any great accuracy. The current flow in the secondary at break is very easily calculated, since it varies inversely as the total resistance in the primary when a coreless coil is employed[1]. The measurements for each recovery curve were confined to the period when the excitability is less than normal. No attempt was made to determine the curve in the period of supernormal excitability[2].

---

[1] This *Journal*, XLIV. p. 112. 1912, and XLVI. p. 389. 1913.

The temperature at which the curves were measured varied between 22·5° and 5° C. For each preparation the recovery curve was determined at three or four (occasionally five) different temperatures, and at the end of each series of measurements a few points on the curve were redetermined to make certain that the condition of the nerve had not changed. As a rule the temperature did not vary more than ·3° C. during the measurement of each curve; the curve was rejected when the variation was greater than ·6° C.

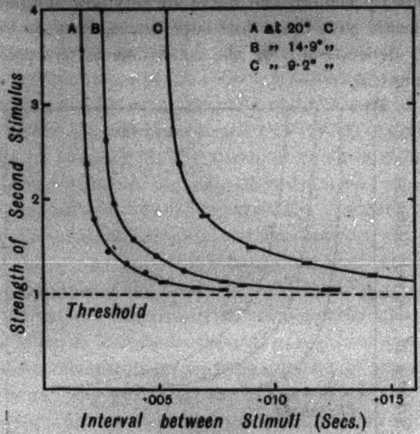


Fig. 2.

Typical records are shown in Figs. 1 and 2. These are constructed on the same lines as those already published by Lucas[3] and Adrian[4]. The abscissæ give the time intervals between the first and second stimulus; the ordinates give the strength of the second stimulus necessary to produce muscular summation at any given interval. The threshold strength (*i.e.* the strength necessary to excite the nerve when it has recovered completely) is taken as unity, and greater strengths are expressed as multiples of it. The threshold was determined at each temperature and before and after the measurement of each point on the recovery curve. The accuracy with which each point was determined is represented as far as possible by the size of the rectangles through which the curve passes. Very often the limits of error are not great enough to be reproduced in this way in a small diagram.

---

[1] **Martin.** *Amer. Journ. Physiol.* xxii. p. 116. 1908.

[2] **Adrian and Lucas.** This *Journal,* xliv. pp. 111 *et seq.* 1912.

[3] *Ibid.* xlvi. p. 486. 1913.

[4] *Loc. cit.*

In Fig. 1 (Exp. 1) the recovery curve was determined first of all at 20° (A), then at 10·7° (C) and lastly at 14·8° (B). In Fig. 2 (Exp. 2) the temperatures were 20°, 15·2° and 9·0°, and the curves were determined in the order B, C, A. All six curves are of the continuous type, and may therefore be taken to give a true measure of the return of excitability at the point stimulated.

An inspection of the curves suggests that changes of temperature affect them in a very simple way; it suggests that the equation of the curve has the same form—$y = F(cx)$, at all the temperatures examined, the only effect of altering the temperature being to change the value of the constant c by which the time intervals must be multiplied. This possibility is tested below in Tables I and II. The first column of these tables gives the strength of second stimulus necessary to excite the nerve at different intervals after the first stimulus. The second, third and fourth columns give the intervals corresponding to these strengths at the three temperatures examined. Thus in Exp. 1 (Table I), at 20° a stimulus 1·5 times as strong as the threshold will excite the nerve if it falls at an interval of ·0028 second after the first stimulus. In other words, the excitability of the nerve has returned to 75% of its normal value after an interval of ·0028 sec. At 14·8° the time taken in recovering to this excitability is ·0051 sec. Thus at the lower temperature the time of recovery is 1·82 times as long as it is at 20°. This number is found in the fifth column, which gives the ratio of the times taken to recover to different excitabilities at 14·8° to the times taken to reach the same degree of recovery at 20°. The sixth column gives the ratio of the recovery times at 10·7° to those at 14·8°. It will be seen that the ratio of the recovery times at two different temperatures is very nearly constant for all degrees of recovery. Whatever the strength of the second stimulus, the recovery time at 14·8° is 1·8 times as long as that at 20°, and the recovery time at 10·7° is 1·49 times as long as that at 14·8. This result holds good in Exp. 2 and in all the other experiments made. The total number of experiments was 11; in three of these the curve which was the last to be determined did not agree very well with the first curve, but this is not to be wondered at in an experiment which may last as long as three hours when five curves are determined (as was the case in these three experiments). There was never any reason to suppose that the form of the curve varies with alterations of temperature between 22·5° and 5° when the condition of the preparation is otherwise constant.

We may conclude then that a change of temperature has no effect beyond that of altering the time scale of the curves; their form remains constant at all the temperatures examined. This result is interesting for several reasons. In the first place it shows that the recovery of excitability is essentially the same process at all temperatures. This we might have expected, but it is satisfactory to have this expectation confirmed. Again, we may speak of the temperature coefficient of the rate of recovery of excitability without defining any particular range of excitability over which the coefficient holds good. The rate of recovery

TABLE I. *Exp.* 1.

Strength of second stimulus required for muscular summation (Threshold strength taken as unit)	Interval between first and second stimulus (in seconds)			Ratio of these intervals	
	A (20° C.)	B (14·8° C.)	C (10·7° C.)	$\frac{B}{A}$	$\frac{C}{B}$
3·25	·00150	·00270	·00410	1·80	1·52
3·00	·00154	·00280	·00420	1·82	1·50
2·75	·00164	·00296	·00440	1·81	1·49
2·50	·00174	·00312	·00460	1·79	1·47
2·25	·00190	·00334	·00490	1·76	1·47
2·00	·00204	·00366	·00540	1·79	1·47
1·75	·00230	·00420	·00620	1·82	1·48
1·50	·00280	·00510	·00740	1·82	1·45
1·25	·00360	·00660	·00970	1·83	1·47
1·15	·00420	·00770	·01150	1·83	1·49
1·10	·00480	·00870	·01280	1·81	1·47

TABLE II. *Exp.* 2.

	A (20° C.)	B (15·2° C.)	C (5·0° C.)	$\frac{B}{A}$	$\frac{C}{B}$
3·00	·00175	·00270	·00560	1·54	2·08
2·50	·00185	·00278	·00583	1·50	2·10
2·25	·00191	·00286	·00600	1·50	2·10
2·00	·00198	·00308	·00645	1·55	2·09
1·75	·00218	·00340	·00730	1·56	2·14
1·50	·00280	·00430	·00890	1·535	2·06
1·30	·00365	·00570	·00170	1·56	2·05
1·20	·00420	·00660	·01400	1·57	2·12
1·15	·00495	·00750	·01520	1·52	2·03

from 25°/₀ to 50°/₀ excitability has the same temperature coefficient as that from 50°/₀ to 75°/₀ and so on. A more important result lies in the fact that the ratio of the times taken to recover to a definite excitability (say 75°/₀) at two different temperatures remains the same whether the times are measured from the moment at which the first stimulus is sent in, or from the moment when the excitability has returned to 25°/₀ or 50°/₀ of its normal value. It follows that the effect of a change of temperature on the rate of recovery of excitability is the same as its effect on the duration of the period in which the nerve is completely inexcitable. In other words, the temperature coefficient of the absolute refractory period is the same as that of the relative. This naturally suggests that all the phenomena connected with the refractory state are really manifestations of one single recovery process. However, the truth of this has yet to be proved, for as a matter of fact the argument from the equality of the temperature coefficients is seriously weakened by the fact that this particular coefficient has a value somewhere between 2·8 and 3·1, and that this happens to be the temperature coefficient of a number of different events which are certainly not connected directly with one another or with the recovery process[1]. Consequently the absolute and the relative refractory periods may very possibly depend on different recovery processes in spite of the fact that they are affected in the same way by a change of temperature. At the same time this fact is of some importance, for many of the phenomena of excitation and conduction in nerve have a much lower temperature coefficient, and there might well have been some difference in the coefficients of the absolute and relative refractory periods.

As regards the actual value of the temperature coefficient as deduced from these experiments, it was stated in the introduction that the least interval for muscular summation could not be taken as a measure of the rate of recovery of the nerve because its length depended on two factors which might vary independently, viz. the rate of return of the local excitability and the conductivity of the nerve between the point stimulated and the muscle. In Figs. 1 and 2 the recovery curves are continuous, and so the conductivity factor does not come into play. Consequently in these two experiments the coefficient might have been measured in this way without fear of error. A good example of the fallacy which the method often involves is shown in Fig. 3. This figure gives the recovery curves of a preparation at four different temperatures, 20°, 15·5°, 12·1° and 8·3°. At the two lowest temperatures the recovery curves are discontinuous, the curve D suddenly becomes vertical at ·0060 sec., and the curve C probably does so at ·0034 sec. Evidently at 8·3° C. some factor comes into play preventing any second propagated disturbance from reaching the muscle if it has been set up earlier than ·0060 sec. after the first. This factor might be either a decrement in conduction between the electrodes and the muscle, or else the presence of a region of nerve at a temperature slightly lower than that of the point stimulated. Such a region would recover more slowly, and consequently a disturbance set up at an early stage in the recovery of the warmer region might arrive at the cold region at a time when it was still unable to conduct at all. This is the more probable explanation, for the cooling was confined to the nerve and did not affect the muscle or nerve ending, and as a rule a nerve does not conduct with a decrement because it is cooled to 8° C. But whatever the precise nature of the factor involved, it is clear that its appearance at low temperatures makes it impossible to compare the least intervals for muscular summation with a view to determining the temperature coefficient of the recovery process. If we do compare these intervals, we obtain a value of 3·66 for the coefficient between 18° and 8°, whereas the value determined from the rate of recovery of excitability is only 2·8. Bazett's value of 3·6 for the coefficient of the intramuscular nerves of the sartorius may be due to

---

[1] Cf. Kanitz. *Ztsch. f. Elektrochemie*, XIII. p. 707. 1907.

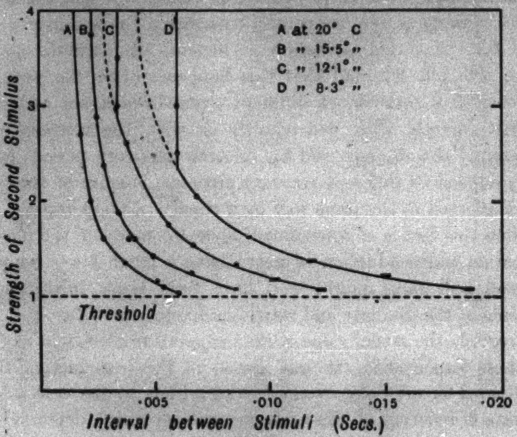


Fig. 3.

much the same causes. However, the two cases are not strictly comparable, as Bazett worked between 5° and 15° instead of 8° and 18°.

The temperature coefficients observed in the present experiments are given in Table III. They are calculated from the time taken by the nerve to recover to half its normal excitability at T° C. divided by the time taken to reach the same stage of recovery at $T+10$°C. The times are measured from the moment when the first stimulus is sent into the nerve. As we have seen, the coefficient obtained in this way will hold good for all stages of recovery, and for the absolute as well as the relative refractory period. The table gives the value of the coefficient between 10° and 20°, and also between 8° and 18° whenever this is possible. When the rate of recovery was not actually measured at one of these temperatures, the required value was found by interpolation. This proceeding is not likely to introduce very much error, as the actual observations were made at temperatures near enough to 10° or 8° to leave little doubt as to the required value.

TABLE III.

	$\frac{\text{Time of recovery to } \frac{1}{2} \text{ excitability at } T^\circ}{\text{Time of recovery at } T+10^\circ}$	
Exp.	$T=10^\circ$ C.	$T=8^\circ$ C.
1	2·75	—
2	2·85	—
3	2·95	2·87
4	2·82	2·80
5	3·05	3·20
6	3·10	3·15
7	2·73	—
8	2·92	3·05
9	2·80	2·91
10	2·88	—
11	2·98	2·88
Mean value	2·90	2·97

The mean value for the coefficient between 10° and 20° is 2·90; that for the lower range of temperature is 2·97. There appears to be some tendency for the value of the coefficient to rise as the temperature is reduced, but this is not always the case. For instance in Exp. 4 the coefficient has the same value (2·82) whether it is measured between 10° and 20°, or between 5° and 15°. Fig. 4 shows the effect of different temperatures on the recovery time to half excitability in this experiment. The curve is evidently logarithmic and not linear, and this result is true of all the preparations examined. Whether this can be taken to imply that the recovery process is essentially a chemical change is a question which has been discussed already in the introduction. No attempt was made to investigate the form of the curve in detail over a wide range of

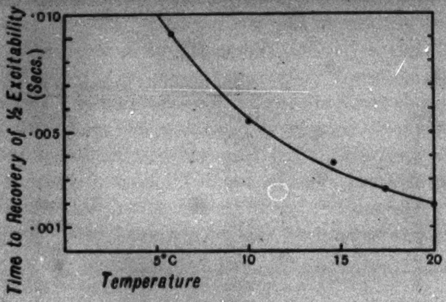


Fig. 4.

temperature, since for purely comparative purposes it is enough to know the value of the temperature coefficient measured between two standard temperatures such as 8° and 18° C.

SUMMARY OF RESULTS.

The rate of recovery of excitability was measured by determining the full "recovery curve" of the preparation. It was found that a change of temperature affects all stages of recovery to the same extent. The temperature coefficient of the absolute refractory period is the same as that of the relative, and the coefficient of the rate of recovery of excitability is the same for all degrees of recovery. This suggests that a single process accounts for all the phenomena of the refractory state. The duration of the refractory period at 10° C. divided by that at 20° C. gives a mean value of 2·90 for the temperature coefficient. The mean value between 8° and 18° is 2·97. The rate of recovery is a logarithmic and not a linear function of the temperature, but it does not follow that the process of recovery is necessarily a chemical as distinct from a physical process.

THE REGULATION OF THE HEART BEAT. By S. W. PATTERSON, H. PIPER and E. H. STARLING.

(*From the Institute of Physiology, University College, London, and the Physiological Institute of the University, Berlin.*)

THE employment of the heart lung preparation has enabled us to study and analyse much more completely than was formerly the case the mechanical performance of the mammalian heart and to obtain clearer ideas of its capacity for work and its power of adapting its work to the mechanical conditions imposed upon it. We have learned that while the inflow is maintained constant, the output from the left ventricle is unaltered by rate of heart beat or by the resistance presented by arterial pressure, and on the other hand, that within wide limits the heart is able to increase its output in direct proportion to the inflow. The study of the respiratory exchanges of the heart lung preparation has shown that the metabolism of the heart increases or diminishes in proportion to the mechanical demands made upon this organ, so that the chemical sources of energy are drawn upon in proportion to its requirements. It is difficult to imagine a more perfectly regulated machine than the heart. In the experiments here described we have endeavoured to determine some underlying principle on which the heart's power of self-regulation may depend, or some elementary property of the tissues making up the heart from which the varied behaviour of the heart under different circumstances can be directly deduced.

In our preparation the heart is free from all nervous control. The question arises whether we can refer to one or a few elementary qualities of the muscular tissue the whole behaviour of the heart, under stress or light work, when efficient or failing, when stimulated or depressed by drug or nerve. The solution of this question can only be arrived at by accurate analysis of the mechanical phenomena of the heart beat under rigorous control of all its conditions. The results of such an analysis, which has occupied us for the last two years, are presented in the following pages. In order to lend interest to the dry facts, curves, and measurements of time and pressure, which we have to record, we propose to reverse the usual order of a physiological communication and to begin by giving the general conclusions to which our experiments have led us. We shall then give the facts on which these conclusions are based, and shall endeavour to show that they are consistent with and confirmatory of the principles which we lay down.

The behaviour of the heart must be the sum of the behaviour of the muscle fibres of which it is composed. In considering the behaviour of contracting muscle, the original conception of Weber is of great assistance,—the conception according to which the muscle during contraction changes into a body with other elastic properties. In the words of A. V. Hill, "excitation causes a setting up of a new state in muscle. At and after this moment the muscle is simply a new elastic body of changed elastic properties and possessing potential energy which can be turned into work, if we desire, but which otherwise will degenerate in a few hundredths of a second into heat." Under the conditions of an ordinary physiological experiment a contracted muscle loaded only by a light lever is shorter than the non-contracted, but can be stretched to the length of the latter by a certain weight, when it will be in a condition of tension. This tension is often regarded as the measure of the power exerted by the muscle when it passes from the resting to the contracted state. Under normal circumstances, *i.e.* in their natural position in the body, muscles may possess any length between extreme contraction and extreme elongation, whether they are in a resting or in an excited condition. The relaxed muscle however requires only a minimal force to extend it to the maximum length possible under normal (*i.e.* the body) conditions, so that it is usual to speak of the different lengths of a contracted and uncontracted muscle, the lengths being in this case those which are impressed on the muscle by a minimal load.

The investigation of the maximum mechanical energy set free in the contraction of a muscle under different conditions of excitation was carried out by Blix (1), who arrived at the important result that this amount was a function of the length of the muscle fibres during the period of contractile stress set up by the excitation. The maximum energy which could be set free by a contracting muscle was measured by the isometric method. We have drawn Fig. 1 to represent the results of one such experiment. In this figure the ordinates represent the initial length of the muscle measured from above, and the abscissæ, the tension developed when the muscle passed into a state of contraction. He found that with increase in the length of the muscle, the tension developed also increased to the degree of maximal extension possible in its natural relationships in the body. To stretch the muscle beyond this length required an ever-increasing initial tension. The contractile stress set up on passing to the excited state increased for a time with increasing initial tension and then diminished, so that the lines in the figure tend to approach one another. **Heidenhain** had already shown that the heat production in muscle was a function of the contractile stress it developed, and **Frank** (2), in his summary of the work on the development of heat in muscular activity, concludes that the essential determining factor is not the tension but the length of the muscle during its state of activity. He states "if we take the length of the muscle as the independent variable, all relationships of heat production to the changes of mechanical state in the muscle can be represented with extraordinary facility." This conclusion has received powerful confirmation lately by the work of A. V. **Hill** (3). He shows, in confirmation of **Blix**, that the shortening of muscle diminishes the heat

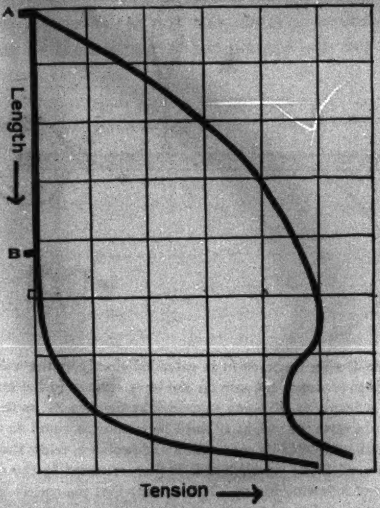


Fig. 1. Curve showing the relation of tension, initial length, and energy evolved in isometric twitch. (Drawn from the results of one of **Blix**'s experiments. *Skand. Arch. f. Physiol.* v, Tab. VI, Fig. 4.)

A = Condition of maximum shortening.

B = Position of maximum length of muscle in the body.

production in so far as the chemically active surfaces are diminished, while lengthening the muscle increases the chemically active surfaces. The longer the muscle therefore (within physiological limits) the greater the amount of chemical energy, heat production, and tension set up when the muscle passes from the resting to the active condition[1].

These results can be transferred unreservedly to heart muscle, making only certain modifications that are necessitated by the anatomical arrangements in this organ. In the first place when the heart muscle is unloaded, *i.e.* when the heart is empty, the length of the relaxed muscle is the same as that of the contracted muscle (apart from slight shifting of the mutual position of the muscle fibres produced by rotation of this organ on its axis). According to the state of filling of the heart, the length of the "resting" muscle may be anything between that of maximal dilatation or lengthening and maximal contraction.

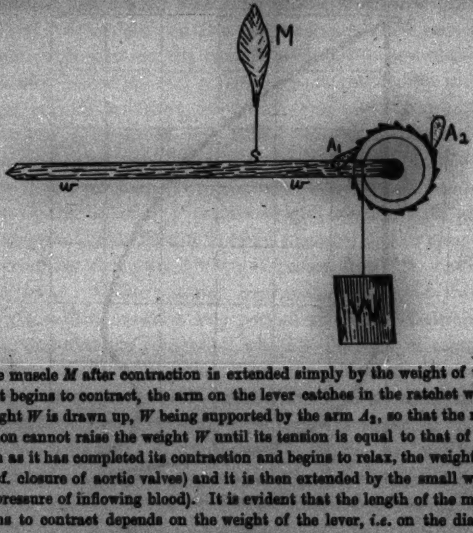


Fig. 2. The muscle M after contraction is extended simply by the weight of the lever w. When it begins to contract, the arm on the lever catches in the ratchet wheel so that the weight W is drawn up, W being supported by the arm A_2, so that the muscle after excitation cannot raise the weight W until its tension is equal to that of the weight. As soon as it has completed its contraction and begins to relax, the weight W is taken off it (cf. closure of aortic valves) and it is then extended by the small weight of the lever (pressure of inflowing blood). It is evident that the length of the muscle before it begins to contract depends on the weight of the lever, *i.e.* on the diastolic load. The extent to which it will contract depends on the tension aroused in it when it contracts and the amount of the weight W which it has to overcome.

---

[1] It seems probable that the evolution of energy in the form of heat continues to increase with increasing length considerably beyond the length which is the optimum for the development of tension.

Another point of difference refers to the question of load. Under the conditions of ordinary physiological experiments, a skeletal muscle is loaded with a certain weight which it raises when it contracts and which extends the muscle when the latter relaxes. In the heart the extending load on the muscle is much less than the load during contraction. The extending load is represented by the diastolic intracardiac pressure and is dependent on the inflow. The load against which the muscle has to contract is represented by the pressure in the aorta. Until the tension exerted by the heart muscle rises to a height greater than that corresponding to this pressure, the contraction of the heart is isometric, and the muscle fibres can undergo no change in length. These conditions, as Henderson (4) has pointed out, can be imitated on a skeletal muscle by attaching to it weights arranged somewhat after the fashion of an "Arbeitssammler," as in the diagram (Fig. 2).

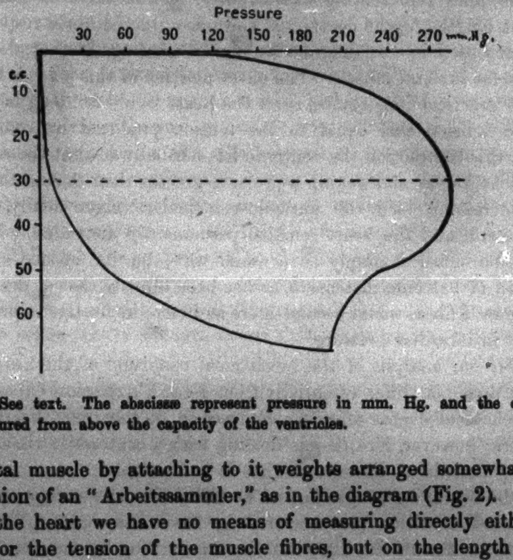


Fig. 3. See text. The abscissæ represent pressure in mm. Hg. and the ordinates measured from above the capacity of the ventricles.

In the heart we have no means of measuring directly either the length or the tension of the muscle fibres, but on the length of the muscle fibres depends the capacity or volume of the heart, while the tension of the muscle fibres determines the intracardiac pressure. If we assume a linear relationship between capacity of heart and length of muscle fibre and between intracardiac pressure and tension of muscle fibre, we can in the muscle extensibility diagram replace length of fibre by heart volume, and tension of muscle fibre by intracardiac pressure under the two conditions of rest and activity. If we do this, we must remember that the linear relationship even if it holds in the contracted heart, must be more and more departed from as the heart becomes distended and its shape more globular (cf. note in last paper(5)).

Taking these facts into consideration, we may draw a diagram for the heart, expressing the relation between volume and pressure, and interpret these relationships in terms of length and tension. The diagram (Fig. 3) is copied from Frank's(6) diagram representing the results of his measurements on the ventricle of the frog. We have however placed numbers which would in our opinion approximately represent the relation of the capacity of the left ventricle to its diastolic and systolic pressures in a healthy dog's heart weighing about 70 gms. The two lines representing extensibility, or the state of tension for different lengths of the contracted and uncontracted fibres respectively, are represented in the diagram as finally meeting, as they do in the diagram for skeletal muscle. The exact position of this point is however of little practical importance, since the heart would fail long before the diastolic tension was equal to the tension produced by contraction. During this contraction the ventricle has normally to shut the auriculo-ventricular valves and get up a pressure greater than that in the aorta. With increasing load the auriculo-ventricular valves finally become incompetent, and the heart remains permanently distended, with each contraction marked simply as a small wave on its surface. In this condition of extreme distension, as has been already shown, the tension of the muscle fibres would become more and more ineffective in producing a rise of intracardiac pressure.

So far our analysis of the mechanical condition of the heart's contraction does not differ essentially from Frank's classical exposition of the mechanical events affecting contraction of the frog's heart(7). In this work, however, Frank was dealing with a contractile tissue which had long periods of diastole, so that it had reached a static condition before the beginning of systole, and he found that the energy set free in the excited condition (as measured by the tension set up) was proportional to the initial, *i.e.* diastolic, tension on the muscle. But in this case it is not easy to decide whether the determining factor is really the initial tension or the initial length of the muscle fibres. Rohde(8), from a study of the perfused mammalian heart and a comparison of its isometric contractions and gaseous exchanges under varying conditions of initial and final tension, has come to the conclusion that in the heart, as in the skeletal muscle, there is a certain volume of the heart at which the greatest development of energy will occur when the heart enters into a state of contraction, whether isometric or isotonic. This optimum volume would correspond to what Blix calls the natural length of the skeletal muscle. As a result of his experiments he makes the same change in Frank's formula for the contraction of the heart that the latter authority had already made for the connection between heat production and other mechanical conditions of skeletal muscle, namely, in place of initial tension he speaks of initial length of fibre or initial volume of the heart. Blix' statement for voluntary muscle could therefore be applied directly to the contracting heart. The proof that this replacement of initial tension by initial length is justified, is not however clear from Rohde's results, but we find the conclusion fully justified in the light of our experiments.

In the mammalian heart we are dealing with an organ which is contracting rapidly and rhythmically, such contraction being a necessary condition for the preservation of its functional activity. The condition of the heart therefore as to both tension and length of its muscle fibres is altering continuously during diastole from one beat to the next.

Although this fact makes the analysis of the experimental results more difficult, the results are instructive, since they enable us to dissociate tension from length as factors in determining the energy evolved at each contraction. In Frank's experiments on the frog's heart the initial length was proportional to the initial tension, so that the augmentation of energy set free on contraction might have been due either to the increased tension or to the increased length. In our experiments we have found that it is length rather than tension which determines the energy of contraction,—a result which might be expected from what we know as to the influence of length on the evolution of energy in skeletal muscles. The proof that it is length rather than tension which determines the contraction of the heart is to be found in a study of the changes which occur at the beginning of diastole. If we take a heart which is contracting vigorously and in which the state of contraction passes off rapidly, with a small inflow we may find that it practically empties itself during systole and then rapidly relaxes. In this case the inflow during diastole is, so to speak, only just sufficient to follow up the relaxing ventricle and the pressure in the ventricle remains approximately zero up to the beginning of the next systole. We may now double the inflow without making it excessive for the heart, so that at the end of diastole the heart, although containing twice as much blood as previously, cannot be regarded as actively stretched by the latter, and the initial pressure at which the heart begins to contract is still indistinguishable from zero. The heart however is fuller than before, its capacity may be doubled although its initial tension is not measurably altered. In this case we find that the tension developed by the contracting ventricle is increased in proportion to the increased filling of the heart.

We thus find no constant connection between the diastolic tension and the succeeding contraction, though as a rule these two quantities will be altered together. But we do find a direct proportion between the diastolic volume of the heart (*i.e.* the length of its muscle fibres) and the energy set free in the following systole.

The law of the heart is therefore the same as that of skeletal muscle, namely that the mechanical energy set free on passage from the resting to the contracted state depends on the area of "chemically active surfaces," *i.e.* on the length of the muscle fibres. This simple formula serves to "explain" the whole behaviour of the isolated mammalian heart,—its movements, powers of adaptation to varying demands made upon it, its behaviour in fatigue and under the influence of its nerves or chemical agencies, such as acid ions or adrenalin.

Let us take two examples. The left ventricle is putting out 600 c.c. of blood per min. against a mean arterial pressure of 80 mm. Hg. The mean arterial pressure is then raised suddenly to 140 mm. Hg. On measuring the total output it is found to be 600 c.c. as before. The heart continues to put out as much blood as it receives, though, as shown by Evans, at a greater expenditure of chemical energy. If we trace out the events occurring at each beat, the mechanism of adaptation is evident. At 80 mm. Hg. pressure the heart at each systole is developing just enough energy to put out say 8 c.c. of blood against a resistance which may vary from 65 mm. Hg. at the beginning of the outflow to 100 mm. Hg. towards the end. The arterial resistance is then suddenly increased, so that it needs a pressure of 110 mm. Hg. to force any blood through it and a mean pressure of 140 mm. Hg. in the aorta to keep up a flow through it at the rate of 600 c.c. per minute. The next heart beat gets up enough pressure to force some blood into the aorta and distend it up to 100 mm. Hg.,—let us say 3 c.c. Here the outflow stops. At the beginning of the next systole there is therefore 5 c.c. of blood more in the left ventricle than at the beginning of any preceding diastole. But the venous inflow is still proceeding steadily at 600 c.c. per minute or 8 c.c. per beat. At the beginning of the next systole the heart therefore contains its original residual blood, *plus* 5 c.c. residual blood from the last contraction, *plus* 8 c.c. which have entered it during diastole. The muscle fibres are therefore longer than before by an amount corresponding to the 5 c.c. extra capacity of the ventricle, and therefore when they enter into the contracted condition, more energy is set free and the tension exerted by the muscle fibres is greater. We may assume that they get up enough tension to drive out blood against a pressure of 130 mm. Hg. The outflow of blood into the aorta through the resistance goes on until the pressure rises to 130 mm. Hg. and then ceases. Let us assume that 6 c.c. of blood are driven out. At the beginning of the next systole the heart therefore contains residual blood, 5 c.c. + 2 c.c. + 8 c.c. It is still more distended than before, the muscle fibres are longer, and more energy is set free on contraction. This may be enough to drive out the whole 8 c.c., which are entering it steadily during diastole, against a mean arterial pressure of 140 mm. Hg. If not, the process of increasing dilatation goes on until the energy of the contracting fibres is just sufficient. The heart then continues to beat at this increased volume and to maintain the original outflow against the greatly raised arterial pressure.

Similar reasoning serves to explain the automatic adaptation of the heart to increased inflow. The energy set free at each contraction of the heart is a simple function of the length of the fibres composing its muscular wall. It is evident that the same reasoning applies to the explanation of the almost instantaneous adaptation of the heart to artificially induced lesions of the valves or to artificial stenosis of the aorta. The output of the heart is a function of its filling, the energy of its contraction depends on the state of dilatation of the heart's cavities.

The meaning of tone and fatigue. Like any other living tissue the heart is unable to keep up a high degree of work for long periods of time, it needs opportunity for recuperation, it shows fatigue. In a skeletal muscle contracting isometrically, fatigue is shown by the fact that, starting at a given length, the change from the relaxed to the contracted state is attended with a smaller production of tension than was the case when the muscle was fresh. We may assume that the liberation of chemical substances responsible for the tension change becomes less either from diminution of their concentration within the cell or deficient building up between each contraction. Or we may regard the chief factor in fatigue as due to some retardation in the diffusion away or neutralisation of the active substances, so that the same concentration at the active surface causes smaller changes of potential and of surface energy. But the relation between the length of the muscle fibre and the tension set up at its contraction remains as before. If the muscle fibre with an initial length of 2 cms. displayed a tension of 50 gms., and when elongated to 3 cms. a tension of 80 gms., a state of moderate fatigue might depress these two figures respectively to 30 gms. and 50 gms. The energy set free in contraction would still be a function of the length of the muscle fibre. In the same way, if the heart wall at a certain condition of filling, produces on contraction a tension equal to 100 mm. Hg., and when it is tired can only raise the intracardiac pressure to 80 mm. Hg., it is merely necessary to lengthen the fibre, *i.e.* to increase the filling of the heart, in order to obtain once more at contraction a tension of 100 mm. Hg. This means that a tired heart *must* dilate in order to carry on the same work as the fresh heart, and we know that this condition actually occurs. If by increasing the supply of blood or oxygen to the tissue, or by diminishing the load, we give the muscular fibre of the heart wall an opportunity to recover, the heart may once more return to its normal size, the evolution of energy per unit length of the muscle fibre at each contraction becoming the same as before. We get here a clue to the meaning of the term *tonus* or *tone* of the heart muscle. This term is constantly employed both in physiological and pathological literature, but it has hitherto been very difficult to obtain a clear conception of what was really meant by the word. When, on opening the chest, the heart is found to be dilated, it is often said to be "lacking in tone," and on the other hand, if the heart is found small and evidently emptying itself completely during systole and dilating only slightly during diastole, its tone is said to be increased. But the state of dilatation of the heart may be merely a question of the amount of blood entering it from the veins. A contracted heart may be in a bad condition and a dilated heart may be in a good condition. It is evident from what we have said above that the word *tone* is properly employed as synonymous with physiological condition or fitness of the muscle fibre, and its measure is the energy set free per unit length of muscle fibre at each contraction of the heart. A good heart, *i.e.* one with a good tone, will carry on a large circulation against a high arterial pressure and nearly empty itself at each contraction, while a heart with a defective tone, as is the case when it is tired, can carry on the same circulation but only when its fibres at the beginning of contraction are much longer, *i.e.* when the heart is dilated. In the latter case the output of blood will be the same as in the former, but both the systolic and the diastolic volumes of the heart will be increased.

Fatigue of the heart may go on to heart failure. This occurs when the dilatation, which is the mechanical result of unchanging inflow and failing outflow and is the automatic means of regulating outflow to inflow, proceeds to such an extent that the tension of the muscle fibres becomes increasingly inadequate in producing rise of intracardiac pressure. The mechanical disadvantage, at which in the dilated spherical heart the skein of muscle fibres must act, finally smashes up the system and the circulation comes to an end.

The investigation of the mechanical conditions of contraction in skeletal muscle involves the measurements of its tension and length under alterations of initial and final load at different temperatures and with repetition of stimulus to the point of fatigue. To carry out the same analysis of the conditions determining the contraction of the heart we must in the first place measure the diastolic and systolic volumes of the heart and its output under varying conditions of inflow, arterial resistance, frequency of heart beat, temperature and fatigue. In the second place we must determine the tension or pressure in each of the heart cavities and during every phase of the heart's cycle under the same conditions.

The first part of the investigation, namely, the determination of the changes of volume of the heart under varying conditions, has been for the most part carried out by two of us at University College, while the investigation of the rapid changes in pressure occurring under the same conditions in the different heart cavities, was carried out in the Physiological Institute of Professor **Rubner**, in the University of Berlin. Shortly before beginning our work in Berlin, we heard from Dr **Hermann Straub** that he had been investigating a similar problem and by somewhat similar methods at Munich. He very kindly sent us a copy of his paper on the subject, which has since appeared in the *Archiv für klin. Medizin*(9). Although the conclusions to which we have arrived differ somewhat from those of **Straub**, we found the knowledge of his results of considerable value. In his paper he gives the same description of the reaction of the heart to increased arterial pressure as that given by **Frank** for the frog's heart, namely, as a reaction to initial tension (Anfangsspannung), an explanation which we regard as true only in so far as the initial tension determines initial length. His account of the reaction to increased inflow is less satisfactory, but this may probably be ascribed to his inability, working single-handed, to control all the conditions of a complicated experiment which in our case required the united efforts of three workers.

Methods. All the experiments were carried out on the heart lung preparation on dogs varying from 4 to 8 kilos. The arrangement of the heart lung preparation was the same as that described in the paper by

Patterson and Starling(9). For the measurement of the changes occurring in the volume of the heart at each contraction, we employed a glass cardiometer similar to that used by Henderson. The mouth of the cardiometer was covered with a rubber membrane in which a hole was burnt of such a diameter that when the cardiometer was placed round the heart, the rubber membrane rested in the auriculo-ventricular groove without exerting too great a pressure on the organ, but at the same time effecting an air-tight closure of the cardiometer. If the membrane were too loose, the error was shown by leakage of the apparatus. If the membrane were too tight, it caused a rise of pressure in the venous manometer attached to the inferior vena cava and might at the same time diminish the output from the heart. It was thus possible to adjust the hole in the membrane until it was of a proper size. Many different methods, including the soap bubble method employed by Hermann Straub(10), were tried for recording the changes in the volume of the air in the cardiometer accompanying each beat of the heart. The problem of registering rapid changes in volume is perhaps the hardest task which can be thrown on the graphic method. We finally adopted as the best instrument the large Albrecht piston recorder, already used by Heer(11) in his experiments. This consists of a brass cylinder of about 50 c.c. capacity, in which moves a very light ebonite piston with no lubricant. (We found the piston moved somewhat more easily if the inside of the cylinder and the outside of the piston were polished with graphite powder.) Some of our tracings of heart volume were made by means of a lever in the ordinary manner, so that the ordinate was the arc of a circle. In most cases, however, in consequence of the difficulty of measuring the time intervals under these conditions, we abandoned the lever and recorded the movements of the piston directly by means of a paper writing point attached to a long light aluminium rod, which was connected below to the piston and was attached above to an isotonically weighted lever, and was maintained vertical by allowing it to move up and down in the groove of a jewelled pulley. The rapidity of the response of this apparatus was sufficient to ensure an accurate reproduction of volume changes of air in the cardiometer on the tracing of the piston recorder. The vibration frequency of the system,—cardiometer, tube and piston recorder—was about 20 per second, but oscillations set up in the apparatus were very speedily damped and no trace of such oscillations are to be seen on our tracings.

A more serious danger in the use of the instrument is its sensitiveness to slight alterations of friction between the piston and cylinder. A small particle of dust falling on the instrument, or any deviation of the piston from the vertical, might be seen to impede the free movement of the piston and make it stick in the cylinder. This danger was more marked with small movements of the piston. In most of our experiments however we were able to assure ourselves by a comparison of the total output of the two ventricles, as determined by the cardiometer tracing, and the total output, as directly measured by receiving the blood flowing through the apparatus into a graduated cylinder, that the apparatus was really giving a correct representation of the volume changes of the heart.

In all experiments we measured the pressure at the opening of the inferior vena cava into the right auricle by means of a water manometer, the pressure in the innominate artery by means of a mercurial manometer, and the strength of the arterial resistance by a manometer connected with the air chamber outside the thin rubber tube forming the resistance. The output of the left ventricle (*i.e.* the total output *minus* the coronary flow) was measured by releasing the T-tube connected with the venous side of the apparatus and clamping the tube flowing into the spiral[1], so that all the blood coming to the apparatus flowed into a graduated cylinder. The time taken to collect 50 or 100 c.c. of blood in this way was measured by means of a stop-watch. The temperature of the blood supplying the heart was measured by a thermometer in the cannula leading from the venous reservoir to the superior vena cava. In some cases the left auricle was also connected by means of a cannula filled with hirudin solution with a water manometer.

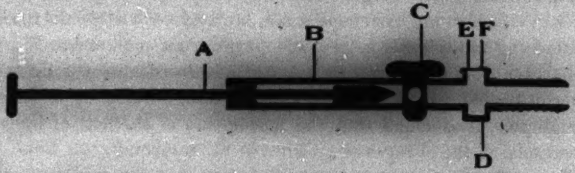


Fig. 4. Diagram to show construction of Piper's trocar manometer. See text.

For the measurement of the rapid changes of pressure occurring in each of the heart cavities,—auricles and ventricles, and in the aorta,—at each beat of the heart, we made use of the methods already employed by one of us (Piper) (12) for determining the form of the ventricular, auricular and arterial pressure variations under a variety of conditions in the whole animal. The only satisfactory method for recording the very rapid changes of pressure which occur in the heart, is the optical method first employed by Frank for this purpose. The method used by us is a modification and simplification of Frank's methods and is similar to that already employed by Straub (18). It is important that the moving mass in such an instrument should be as small as possible and the vibration frequency of the instrument as rapid as possible. The instrument we used consisted of a tube *B* 6 cms. long and 3·5 mm. bore, in which a stilet *A* fitted so that it could slide easily up and down. 3·2 cms. from the lower end is a tap *C* bored out so that in one position it permits the free movement of the stilet, and in the other absolutely closes the tube. Underneath the tap the trocar is widened out so as to form a small cylinder or tambour at right angles to the axis of the trocar. One end of this drum *D* is closed, over the other end is tightly stretched a thick rubber membrane *E*. This membrane forms a manometer, being slightly bulged out with every rise of pressure. The movements, which are extremely small, are rendered visible and recorded by attaching to the lower edge of the membrane a small mirror *F*, 1 mm. in diameter. By this mirror an image of a Nernst filament is thrown on to a slit behind which sensitive paper is moved along by means of a kymograph. It is found that the movements of this slit of light are proportional to the changes of pressure in the tambour.

---

[1] See the illustration in the paper by Patterson and Starling. This *Journ.* XLVIII. p. 357. 1914.

To use the apparatus a purse string suture is put round a spot on the anterior surface of the right or left ventricle. The open end of the manometer with the stilet pushed to the end is then plunged through the wall of the heart in the middle of the ring of suture. The two ends of the purse string suture are then tied tightly so as to lie in one of the grooves with which the lower end of the trocar is provided. The action of the heart is not disturbed at all by this procedure. The stilet is then withdrawn and blood follows. It comes out of the free end of the tube, displacing all air bubbles in the apparatus. The tap is then turned, and we have then a manometer of extremely high efficiency connected directly with the cavity of one of the ventricles. A thinner membrane is generally employed for taking similar changes of pressure in the right ventricle than for the left ventricle. Similar thin membranes were used for recording the pressures in the auricles, the cannula in this case being tied into one of the auricular appendages. In all our experiments the pressure was registered simultaneously in two chambers of the heart, generally right auricle and ventricle or left auricle and ventricle. In a few cases the pressure was measured simultaneously in the left ventricle and in the aorta, and in one experiment the pressures were registered simultaneously in the right ventricle and in the pulmonary artery, a T cannula being introduced into the latter. At the end of the experiment the manometers used were calibrated by determining the extent of the excursion of the spot of light under given variations of pressure. The cannulæ were fixed rigidly in position by iron bars and clamps attached to each side of the table. It is evident that the slightest shifting of either of the manometers would cause an alteration of the zero point and render it difficult, if not impossible, to compare two tracings following one another, taken under different circumstances. In order to carry out such comparisons we adopted two methods. In the first place in the majority of experiments tracings of two successive observations were taken on the same piece of sensitive paper, the drum being allowed to revolve once (first observation), and then when the conditions were changed, it was allowed to revolve a second time, so that no shifting of the paper could occur. As an additional safeguard against any possible shifting of the apparatus or of the manometers we arranged in all our later experiments to have a third spot of light recorded on the tracing. This spot was reflected from a mirror on a rod firmly attached to the same clamps which were holding the manometers in position. If in two successive records on the same length of paper the second record of the fixed spot was identical with the first record, we could be certain that no shifting had occurred in any part of our apparatus. By this means we were able to get accurate records of the rapid pressure changes in the heart cavities and aorta under every condition of inflow up to the maximal, of arterial resistance up to the maximal, at rates varying from 60 to 240 per minute, under stimulation of the vagus, under the action of carbon dioxide and adrenalin, and in every case the pressure in the inferior cava, the output, the average arterial pressure maintained, and the temperature at which the heart was contracting.

In order to correlate these results with those obtained on the volume of the heart by the use of the cardiometer, it was necessary to carry out some experiments in which the volume and pressure changes were recorded simultaneously. For this purpose a method was devised by one of us (Piper).

Our methods of registering rapid changes of pressure are now fairly perfect. But the same cannot be said for our methods of registering rapid changes in volume. We therefore adopted the plan of converting the volume change into a pressure change. A brass cardiometer (*C*) was made with an oval slot in the side wall connecting with the wide orifice into which the heart (*H*) was inserted (see Fig. 5). Both the mouth of the cardiometer and the adjoining oval orifice were surrounded with a lip over which a rubber membrane could be tied. A brass flanged circular ring (*R*) of the same diameter as the circumference of the mouth of the cardiometer was also made. Over this ring a rubber membrane was stretched, and an oval hole burnt in it of a proper size to fit closely over the auriculo-ventricular junction. The ring was then pushed over the ventricles, until the rubber membrane rested in the auriculo-ventricular groove.

A purse string suture was then loosely inserted in the anterior surface of the cavity which it was desired to investigate. The manometer *M* with trocar was pushed through a rubber membrane which was then securely tied round the stem of the manometer. The trocar was then plunged into the heart and tied in by means of the purse string suture. The cardiometer was now passed over the heart, so that the manometer projected through the slot. The rubber membrane on the manometer was then tied tightly by means of a wire passed round the groove surrounding the slot, so that this was closed and made air-tight. Finally the edges of the rubber membrane affixed to the ring were pulled over the cardiometer, and secured by a copper wire in the groove surrounding the edge of the cardiometer.

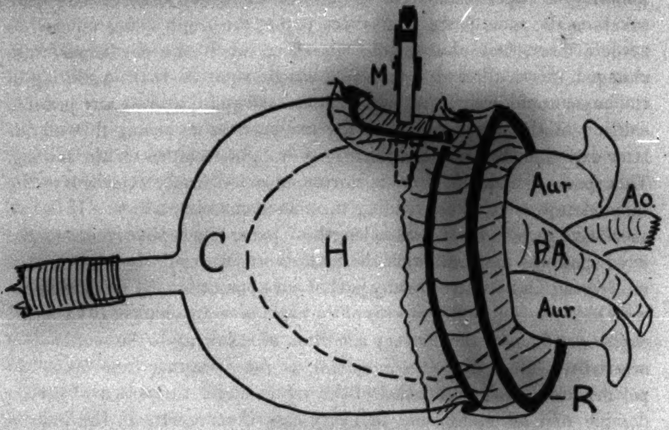


Fig. 5. See text.

The tube at the end of the cardiometer was then connected by thick rubber tubing with a large rubber tambour 12 cms. in diameter. It was found by trial that 50 c.c. of air could be forced into this tambour and produce a rise of pressure amounting to only 20 mm. H_2O. The smaller oscillations of volume, such as would occur as the result of the heart beat, would produce a change of pressure in the manometer of two or three mms. H_2O. To record these small changes of pressure a side tube led from the big tambour to a small glass funnel about 1 cm. in diameter, over the mouth of which a thin rubber membrane was stretched fairly tightly. This membrane formed an efficient manometer for the measurement of the small changes of pressure occurring in the big tambour at each heart beat. The changes of pressure were proportional to the changes of volume and could be recorded optically by means of a tiny mirror attached to the edge of the rubber membrane. In our actual experiments, in our endeavour to avoid any possible hindrance to the action of the heart by changes of pressure in the big tambour, the membrane was stretched a little too loosely, so that the oscillations of our optical cardiometer, as registered on the tracings, were only small. They present also secondary oscillations probably due to the system,—air *plus* rubber membrane closing the large tambour. This could probably be avoided by a slight modification of the apparatus. Since they did not prevent a comparison of the changes of volume with the changes of pressure nor render difficult the reading of the volume curves, we did not trouble to find means of getting rid of them.

I. THE REACTION OF THE HEART TO VARYING ARTERIAL RESISTANCE.

It has been shown that the heart is able to put out a constant amount of blood with very wide variations in the arterial pressure, provided only that the venous inflow is constant. Our first aim was to investigate the changes in the volume of the heart and in the pressures in its various cavities associated with variations in the arterial resistance, the venous inflow being kept constant at a low, medium or high level.

(*a*) *Volume changes of the heart.* These were determined cardiometrically according to the method already described. In Figs. 6 and 7 we show the effects of a sudden increase and diminution of the arterial resistance,—in Fig. 6 with a moderate inflow and an output at each beat of about 5·5 c.c., and in Fig. 7 with an output of about 10 c.c. Both tracings were taken from a dog of 5·15 kilos., with a heart weighing 67 gms. The piston recorder was inverted, so that systole produced a movement of the lever in the upward direction. In Fig. 6 the pressure maintaining the artificial arterial resistance was altered from

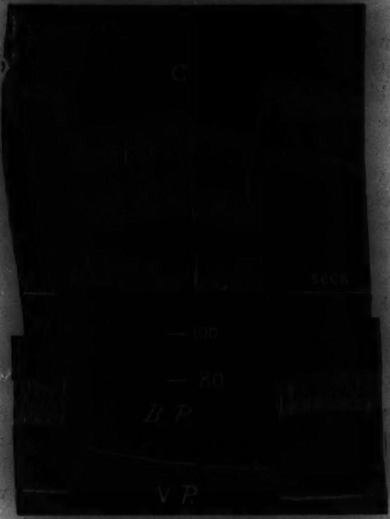


Fig. 6. Effects of rise of arterial resistance on the volume of the heart. (Curves in Figs. 6 to 11 to be read from left to right.)

C, cardiometer tracing; B.P., arterial pressure; V.P., venous pressure. The data of the experiment are as follows:

T.	A.R.	B.P.	V.P.	Rate in 10″	O.P. in 10″
35° C.	40	68	65	22	74
35	103	128	90	22	—
35	42	75	55	22	—

In this, as in all other shortened protocols of the experiments,

T = temperature of blood flowing into the heart.

A.R. = the artificial resistance, as measured by the pressure in the air space surrounding the thin rubber tube in mm. Hg.

B.P. = the arterial blood-pressure as measured by a mercurial manometer connected with the side branch of the cannula in the innominate artery in mm. Hg.

V.P. = the pressure in the inferior vena cava close to the heart, measured in mm. H_2O.

Rate = number of beats in 10 seconds.

O.P. = output in c.c. per 10 seconds, as measured on the peripheral side of the artificial resistance.

40 to 108, then it dropped to 104, and then was suddenly lowered to 42. The mean arterial pressure as measured in the innominate artery was 75 mm. Hg., with a resistance of 42, and 128 mm. Hg. with a resistance

Fig. 7.

Fig. 8.

Fig. 7. Effect of increased arterial pressure on the volume changes of the heart with a large inflow (154 c.c. per 10″).

T.	A.R.	B.P.	V.P.	Rate	O.P. 10″	O.P. per beat calc.	O.P. per beat observed
35° C.	60	98	125	23	154	6·7	10
35	100	128	145	23	—	—	10
35	60	95	120	23	—	—	10·25

Fig. 8. Continuation of same experiment as Figs. 6 and 7, showing volume changes of the heart with a big inflow when the arterial pressure was raised to a height too great for the capacity of the heart.

T.	A.R.	B.P.	V.P.	Rate	O.P. 10″	O.P. per beat calc.	O.P. per beat observed
35° C.	60	95	120	23	154	6·7	9·5
35	96	126	145	23	—	—	9·5
35	144	170	200	23	—	—	9·0

of 103. The pressure in the inferior vena cava was 90 mm. H_2O at 128 and 55 mm. H_2O at 42.

It will be seen that the course of events corresponds exactly to the theoretical case described in the introduction to this paper. On raising the arterial resistance from 40 to 103 the systolic volume increases, *i.e.* the heart does not empty itself so effectively as before. The diastolic volume therefore increases as well, and this increase of systolic and diastolic volume proceeds for eight beats. The heart then takes up a new level in which its systolic volume is practically identical with its previous diastolic volume, and at this level it continues to empty itself as effectively as before. The output, as measured by the cardiometer excursion, is in fact somewhat increased, namely, from 4·6 to 5 c.c. On dropping the arterial resistance again from 103 to 42, the arterial pressure drops and the volume of the heart undergoes an inverse change. Over a series of seven beats it empties itself more effectively before taking up a level which is nearly constant. It does not in this case return precisely to its previous volume but remains somewhat dilated. Fig. 7 shows the same mode of adaptation of the heart to changing arterial resistance when the inflow and output are considerably larger,—10 c.c. per beat. The arterial resistance was raised from 60 to 100, then dropped again to 60. The corresponding arterial pressures were 98, 128, and 95 mm. Hg., and the pressures in the inferior cava, 125, 145, and 120 mm. H_2O. The heart rate was 23 in ten seconds. Here again the heart reacts to a rise of arterial pressure by an increase in its mean volume, taking about five beats to change from one volume to the other. This behaviour of the heart to alterations of arterial resistance is of constant occurrence and shows that the reaction of the heart to alterations in arterial resistance is always accompanied by changes in its mean volume, and that the adaptation to a rise of pressure is effected by the heart dilating until its muscle fibres are of such a length that at their contraction they produce sufficient tension to overcome the increased resistance.

It is evident that this power of adaptation must have its limit, and the limit will be reached when the dilatation of the heart proceeds to such an extent that the muscle fibres work at too great a mechanical disadvantage in producing a rise of intracardiac pressure. This failure of the heart can occur either with a small or a large inflow, only the failure will occur at a lower arterial resistance with a large than with a small inflow. In Fig. 8 we get the effect of failure of the heart with a large inflow, corresponding to about 10 c.c. per beat when the pressure was raised from 96 to 144, a pressure which the heart easily overcame when the inflow was lower. It will be seen that the heart loses ground with each beat, so that it finally contains at the end of systole two or three times as much as it did at the lower pressure. In order to save the heart the arterial resistance was quickly lowered before the dilatation had reached its maximum.

In considering the causation of the gradual changes in the mean volume of the heart we have to take into account not only the mechanical conditions of inflow and arterial resistance but also the physiological state of the heart muscle. This latter will tend to deteriorate steadily during the course of an experiment, and the more rapidly the greater the demands made on the functional capacity of the heart. But this physiological condition—what we have previously defined as *tone*—is itself largely dependent on the supply of oxygen, and possibly food material, to the heart muscle by means of the coronary circulation, which in its turn undergoes alterations in consequence of changes in the mechanical conditions of the heart. As has been shown previously (14), the coronary flow is markedly influenced by the arterial pressure and may for instance be more than doubled by a rise of arterial pressure from 60 to 120 mm. Hg. Added to this mechanical influence we have the effect of metabolites, produced by the heart muscle itself and formed in large quantities when the heart is inadequately supplied with oxygen or subjected to an undue strain. This influence of mechanical conditions on the coronary circulation affects indirectly the physiological state of the heart muscle. Thus, a rise of arterial pressure from 60 to 120 mm. Hg., will at first cause a dilatation of the heart as already described, until the muscle fibres take up a new length at which they can do the work required by them. But at the higher pressure there is a marked increase in the blood supply to the heart muscle. The improved nourishment of the muscle causes an improvement in its physiological condition, and this improvement shows itself by an increase of the tension set up at each degree of contraction in the muscle fibre and therefore a diminution in the length of the muscle fibre necessary to produce a certain tension. We therefore find that the primary dilatation of the heart is followed by a slow recovery, as shown by a diminution of its mean volume. The reverse is also true. When the arterial resistance is suddenly diminished, the heart empties itself more completely and beat by beat returns to a smaller mean pressure. But now the blood supply to the muscle is also diminished and the heart begins to lose ground, so that the primary rapid diminution of volume is succeeded by a slow dilatation. These conditions are illustrated in Fig. 9, showing the effect of a series of changes of pressure, first in the upward and then in the downward direction. With an arterial resistance of 116 and an arterial pressure of 144 mm. Hg. there is a well marked recovery from the primary dilatation, which then gives place to a slow secondary dilatation, which we may regard as part of the gradual diminution of the functional capacity of the heart muscle which proceeds during the whole survival period of the heart, and more rapidly with inadequate coronary flow. It is this diminution in functional capacity which is responsible for the fact that on lowering the artificial resistance at the end of the tracing first to 70 and then to 28, the volume of the heart does not return to the same level as it had at the beginning of the tracing with an arterial resistance of 40 and 66. This two-fold effect of

Fig. 9. Effect of raising and lowering the arterial resistance on the volume and output of the ventricles.

T.	A.R.	B.P.	V.P.	Rate	O.P. 10"	O.P. per beat calc.	O.P. per beat observed
35° C.	40	70	85	23	110	4·8	6·0
35	66	95	105	—	—	—	6·75
35	98	118	120	—	—	—	6·75
35	116	142	138	—	—	—	7·0
35	70	105	103	—	—	—	6·0
35	28	58	75	—	—	—	5·75

a rise or fall of arterial pressure was described by Anrep (15) as an attempt of the heart to return to its proper volume. The factors we have just mentioned give a more satisfactory explanation of the phenomenon.

It may have been noticed that in some of these curves the excursion at each beat, *i.e.* the output at each beat as measured by the cardiometer, is somewhat larger at the higher pressure than at the lower. This is due to the fact that the increased flow through the coronary vessels is added to the ordinary inflow and outflow of the heart. With a small inflow and a high arterial resistance, the greater part of the outflow may pass through the coronary arteries. This summation of ordinary inflow and coronary inflow is well illustrated in the experiment of which a record is given in Fig. 10. In this case we are dealing with a minimal inflow, only 160 c.c. per minute. On raising the arterial resistance from 40 to 90 the arterial pressure rises very slowly, and there is at the same

Fig. 10. Marked influence of changes in coronary circulation on the total output of the ventricles with high arterial pressures.

T.	A.R.	B.P.	V.P.	Rate	O.P. 10″	O.P. per beat calc.	O P. per beat observed
35° C.	40	60	27	22	26	1·2	1·75
35	90	100	47	—	—	—	2·25
35	140	152	75	—	0	0	2·5 4·5
35	56	80	35	—	—	—	2·0

time a slow increase in the volume of the heart. So small an amount of blood was coming into the heart between each two beats that it did not put out enough to raise the arterial pressure quickly. The output, as measured on the diastolic side of the peripheral resistance, fell therefore to 0, and the blood leaving the heart went chiefly through the coronary vessels, and what was left over was expended in distending the arterial tubes. The arterial resistance was then raised to 140. We notice in the tracing the same slow increase in heart beat. The arterial pressure rose also slowly and then attained a constant level, but there was *no output* as measured on the distal side of the peripheral resistance. Judging from this alone we might have said that the heart was putting out no blood, but a glance at the cardiometer tracing shows us that the output of the heart was double what it had been at the beginning of the experiment. The whole of the blood which it put out was however passing through the coronary vessels, distending the aorta at each beat and leaking rapidly away from this through the coronary vessels, so that none was left over to pass through the peripheral resistance. In this experiment we were dealing with an exaggeratedly small inflow and with an exaggeratedly large arterial pressure, but it serves to illustrate the important changes occurring in the coronary circulation with alterations in the mechanical conditions of the heart beat, and a possible source of confusion when we compare cardiometer records of output with the output as measured by the flow of blood through the arteries and veins of the systemic circulation. The smaller the inflow of blood and the larger the arterial resistance, the more will the output, as measured by the cardiometer, exceed the output as measured directly by the flow through the arterial resistance.

(*b*) *The effect of variations in the arterial resistance on the volume changes of the heart at each beat.* The course of the volume curve at each beat may be studied by taking cardiometer tracings on a rapidly moving drum. In Fig. 11 we give a series of such tracings taken from one experiment which may be considered as typical. The details of the conditions under which each curve was taken are given in the accompanying table.

In the tracings from 1 to 4 the arterial pressure was raised by steps from 84 to 190 mm. Hg., the inflow being maintained constant at a considerable height, namely, about 720 c.c. per minute. In curves 5 to 7 the arterial pressure was reduced again by steps to 46 mm. Hg. On examining first the systole we see that in each case it can be divided into three parts. The first part is represented by a quick descent, corresponding to a rapid outflow of blood from the ventricles. In the second part the outflow becomes much smaller or even stops,—corresponding to the velocity minimum in the pulse curve recorded by Frank. The third part varies in the different tracings. With a low arterial pressure the outflow becomes more rapid again at a point which probably corresponds to a falling arterial pressure and the descent of the so-called systolic plateau. As the pressure is raised two changes are noticed in the form of the curve. The first part of the curve alters in slope, so that the diminution of volume is more rapid at 140 mm. Hg. arterial pressure than at 84 arterial pressure. The chief effect of the rise of the arterial pressure is however on the third part of the curve. In the curves taken with pressures of 140 and 190 mm. Hg. there is practically no further fall in the third part of the curve, so that this part of the curve runs parallel with the abscissa. This behaviour of the curve can only be interpreted by assuming that at this period of the ventricular contraction practically no blood is leaving the ventricles.

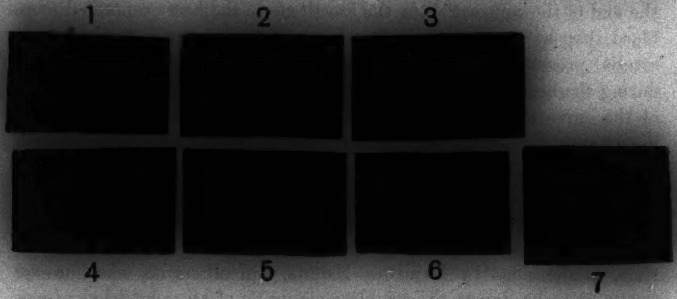


Fig. 11. Cardiometer tracings on a rapidly moving drum, showing influence of varying arterial pressure.

Dog 4·6 kilos; heart 52 gms. Cardiometer writing vertically; systole downwards. Venous supply maintained constant and arterial resistance varied.

	A.R.	B.P.	V.P.	O.P.	Pulse rate
1.	60	84	45	115	26·5
2.	80	110	62	115	26·5
3.	110	140	80	111	26·5
4.	150	190	110	108	26·5
5.	70	106	60	115	26·0
6.	18	50	50	116	26·0
7.	0	46	45	118	26·0

At the side of curves (1) and (2) is given the calibration of the recorder, each division corresponding to an output of 5 c.c.

The flattened systolic curve is still more marked when the inflow of blood is small. When observed with a small inflow and a small arterial pressure it may mean that the ventricles actually empty themselves at each contraction, so that the period of outflow of blood from the ventricles necessarily comes to an end before the contraction of the muscle gives place to diastolic relaxation. This cannot however be the explanation of the flat top observed under the conditions in which the curves in Fig. 11 were taken. From our study of the volume changes, as recorded on a slowly moving drum, we know that the heart has been continually expanding as the arterial resistance was raised, so that at the end of the contraction in the fourth curve the heart contained more blood than at the end of diastole in the first curve. With these high arterial pressures therefore the greater part of the outflow may occur during the beginning of the ventricular systole. During the latter part of the curve the ventricular muscle has maintained a tension which so nearly approximates to that in the aorta that practically no outflow of blood occurs. There seems therefore to be some justification for the old idea that the latter portion of the systole of the ventricles represents a "Verharrungszeit" or "rückständige Kontraktion" in which the ventricle remained contracted without forcing out any blood. This time, as described by Landois, Moens, and others (16), was however supposed to be due to the complete emptying of the ventricle and not, as we see here, to a simple holding up of aortic pressure. It must certainly be quite uneconomical and probably is only obtainable under such artificial conditions as those ruling in our experiments, where we can control factors independently which under normal circumstances of the body vary together. The change in the slope of the first part of the systole which accompanies increased arterial resistance, may be due either to a more rapid development of tension by the contracting muscle fibres, or to the fact that the dilatation of the heart which we have seen to accompany increased arterial resistance, has, so to speak, taken up the slack, so that the contraction of the muscle has a more immediate influence in raising the intra-ventricular pressure.

The diastolic part of the curve, during which the ventricles are being filled, does not present many features worthy of note. The amount of blood flowing in at each systole was the same throughout the experiment. The only difference between the filling of the heart at the high and the low pressure lies in the fact that the venous pressure, and therefore the distending force, is greater when the arterial pressure is high than when it is low. This is probably responsible for the slightly increased steepness of slope in curve No. 4 as compared with curve No. 1. In each case the increased rapidity of inflow with increased volume of the ventricles, due to contraction of the auricles and emptying of their contents into the ventricles, is well marked.

(c) *Pressure changes accompanying the reaction of the heart to variations in arterial pressure.* Whereas the diastolic filling of the heart determines the subsequent contraction, the pressure changes in the heart, as the result of the contraction, are responsible for the changes in volume that we have just studied. We therefore investigated the influence of alterations in the arterial resistance on the pressures in the different cavities of the heart, in each experiment making two simultaneous determinations, namely, (1) the left ventricular pressure with cardiometer tracing, (2) simultaneous records of pressure in the left ventricle and aorta, (3) simultaneous records of pressure in the left ventricle and left auricle, (4) simultaneous records of pressure in right ventricle and right auricle.

In the same experiment we investigated the influence of varied filling of the heart as well as of variations in rate as brought about by stimulation of the vagus or by differences of temperature. The influence of these various conditions on the form of the endocardiac pressure curves in the various cavities of the heart, we shall deal with more fully in a subsequent paper. Here we wish to draw attention merely to the experimental data which throw light on the questions raised in the introduction to this paper.

The influence of changes in the arterial pressure on the volume of the two ventricles and on the pressure in the left ventricle is shown in Figs. 12 and 13. In Fig. 12 the pressure was raised from 68 to 128, the inflow being maintained constant at about 900 c.c. per minute. The tracings at the two different pressures were taken on the same piece of paper so as to allow of an accurate comparison of the tracings under the two sets of conditions. In Fig. 13 the first tracing was taken with an arterial pressure of 132, and the second one with an arterial pressure of 52. It will be noticed that in each case the cardiometer readings show the changes of volume with rise in arterial pressure which have already been described. Both systolic and diastolic volumes increase with rise of pressure and diminish with fall of arterial pressure. In comparing the curves of intra-ventricular pressure, the first thing to note is that, as we should expect, the ventricular pressure rises higher when the arterial resistance is raised. The most interesting point is perhaps however the difference in slope of the rising part of the curve, the pressure increasing more rapidly when the arterial pressure is high than when it is low. Thus in Fig. 13 the ventricle took ·037 second to raise the pressure in its interior 100 mm. Hg. at 130 mm. Hg. arterial pressure. On reducing the arterial pressure to 52 mm. Hg., the same rise of endocardiac pressure was not accomplished under ·074 second. This alteration in the rapidity of rise of pressure is evidently associated with the change in the steepness of the systolic part of the cardiometer tracing, which is also evident in the cardiometer curves photographically registered in Figs. 12 and 13. It is difficult to decide whether this should be interpreted as a more rapid setting up of tensile stress in the contracting muscle fibre, or as the simple mechanical result of the stretching of the heart muscle and therefore less slack to be taken up at the beginning of contraction. We are inclined to ascribe greater probability to the former of these two explanations.

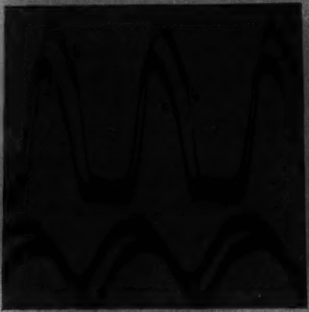


Fig. 12

Fig. 13

Fig. 12. Pressure in left ventricle and of volume of the two ventricles. Curve to be read from right to left.

Dog 6·5 kilos; heart 70 gms.

	T.	B.P.	V.P.	O.P.	Rate	Maximum endo-cardiac pressure mm. Hg.	Time to get up 100 mm. Hg.
a.	37° C.	68	88	153	25·5	183	0·083"
b.	36·8	128	110	153	26	174	0·047

Fig. 13. Same experiment as Fig. 12. Read from right to left.

	T.	B.P.	V.P.	O.P.	Rate	Maximum endo-cardiac pressure mm. Hg.	Time to get up 100 mm. Hg.
a.	36·8° C.	132	128	153	26	178	0·037"
b.	36·7	52	86	153	26	126	0·074

Still more important as regards the mechanical effect of the ventricular contraction in driving blood out into the aorta is the influence of the rise of pressure on the duration of the curve. The duration of the ventricular contraction may be measured by determining the time between the beginning of the rise and the point at which the aortic valves close. In every case increased arterial resistance caused an increase in the duration of the period of contractile stress of the heart. This is quite evident in Figs. 12 and 13 but is still more marked in Fig. 14, where the difference of arterial pressure between the two tracings is greater. In Fig. 12 the duration of the ventricular contraction with an arterial pressure of 68 mm. Hg. was ·1835″ or 48·5 °/₀ and at 128 mm. Hg. ·19″ or 50 °/₀ of total cycle. In Fig. 14 the relative durations of the ventricular contractions at 68 and 166 mm. Hg. were ·224″ or 46 °/₀ and ·262 or 54 °/₀ respectively. In the case of skeletal muscle it has been shown that hindrance to shortening applied during the period of rise of contractile stress increases the total metabolism of the muscle, as judged by its heat production, and also the tension set up in the muscle or the mechanical effect of its contraction. If the extending

Fig. 14. Simultaneous record of left ventricular pressure and ventricular volume. Read from right to left.

Dog 9·5 kilos (pregnant); heart 49 gms.

	T.	B.P.	V.P.	O.P.	Rate
a.	36·2° C.	68	34	88	21·5
b.	36·2	166	66	88	20·5

During the isometric period the time of equal rise of pressure in *b* and *a* is in the proportion of 5 to 9.

force is applied to the muscle at the end of this short period, it has no influence on the metabolic processes of the muscle. The heart muscle appears to differ from voluntary muscle, *e.g.* the skeletal muscle of the frog, essentially in the time relations of the excitatory process.

The chemical change at the surface of the ultimate fibrils, which is responsible for the production of the contractile stress, is not limited to two or three hundredths of a second, as in skeletal muscle, but lasts throughout the whole of the contraction, *i.e.* nearly as long as the mechanical response. The amount of energy set free therefore during each contraction of the ventricles will be determined not merely by the initial length of the fibres composing its walls, but by the length of these fibres throughout the period of contraction. We have already seen that when the arterial resistance is raised, the volume of the heart is permanently greater both in its systolic and diastolic position than its volume with a lower arterial pressure. During the whole of this time therefore the influence of length of muscle fibre will be potent in increasing the mechanical stress set up in the muscle. It is, so far as we know, a new fact, and at any rate one of considerable importance, that this condition increases not only the contractile stress set up during contraction, but also the duration of the excitatory state. As the arterial pressure is raised therefore, we get a marked alteration in the relative durations of systole and diastole respectively. The rhythm of the heart is unaltered, but the systole is prolonged and the diastole correspondingly shortened. This is especially marked in the experiment from which Fig. 14 is taken, where the diastolic period on raising the blood-pressure from 68 to 166 mm. Hg. is diminished by 20 °/₀, namely from ·261″ to ·223″.

There is another feature in the diastolic part of the curve to which we would particularly draw attention. It will be noted that the fall of pressure occurs about as rapidly as the rise, the closure of the aortic valves being probably indicated by the thinning out of the descending part of the curve near its commencement. The pressure falls then rapidly to the zero line and then in most cases rises gradually but very slightly during diastole. (In certain cases the pressure may remain at approximately zero during the greater part of diastole.) In both these tracings the pressure at the end of diastole is approximately the same at the high arterial pressure as at the low arterial pressure. This signifies that the only difference in the condition of the ventricular muscle at the beginning of the systole is one of length and not of tension. The initial tension of the heart muscle is approximately the same under the two sets of conditions, though a reference to the cardiometer curves below the endocardiac pressure curves shows that there is a considerable difference in the diastolic volume of the heart and therefore in the length of the muscle fibres. This constancy of initial tension with varying arterial resistance is of course not invariably found. If the inflow is larger or the heart more dilated to start with, or the rise of arterial pressure excessive, we get a marked difference in the diastolic pressure with difference in initial filling, and in fact we shall expect the diastolic tension to increase with diastolic filling. But cases such as those recorded in Figs. 12 and 13 are of special importance since they enable us to differentiate between changes in tension and changes in length as factors determining the amount of energy set free in the contraction of the heart muscle. We see from these tracings that an invariable condition of increased contractile stress is increased initial length of the muscle fibre. This may be accompanied or brought about by increase in the initial tension of the muscle fibre, but the two conditions are not invariably connected, and we shall find later other cases in which length varying without changes in tension has brought about its proper effect on the strength of contraction of a muscle.

Fig. 14 furnishes an example in which diastolic tension and diastolic length varied together. The change in arterial pressure was here considerable, namely, from 68 mm. Hg. to 166 mm. Hg. We observe the same changes in the systolic part of the curve which we have already recorded—rise of maximum pressure, rise of point of opening of aortic valves, increased steepness of rise of pressure, increased duration of contraction. In the diastolic part of the curve however there is a well marked difference between the two tracings, the diastolic pressure or initial tension at the beginning of systole being considerably higher at the high arterial pressure than at the low arterial pressure.

In Fig. 15 we give simultaneous records of the ventricular pressure as measured in the left ventricle and of the aortic pressure under two conditions, namely, at an arterial pressure of 78 and at an arterial pressure of 156, with a large inflow and output, namely, over 1300 c.c. per minute. The aortic pressure curve at the low pressures resembles closely that given by Frank for the normal animal and presents a well marked dicrotic elevation, followed by two or three oscillations. The rise in the curve immediately preceding the opening of the aortic valves is probably due to a reflected wave from the artificial arterial resistance. The great difference in level between the curves at high and low arterial pressures respectively is due to the fact that the diastolic pressure could not sink below the minimum represented by our artificial resistance. We observe in the systolic part of the intraventricular curve the same increased steepness of slope as with the higher pressure, to which attention has already been called. We would call attention especially however to the diastolic part of the curve. Here it will be noticed that although blood is flowing into the ventricles during the whole of diastole, the pressure does not rise, the inflow of blood evidently being not more than sufficient to follow up the relaxation of the ventricle. This is the case even with the big inflow of 1300 c.c. per minute. There is however a difference of initial tension in the ventricular cavities at the beginning of systole, the tension being higher with the higher arterial resistance. The contraction of the ventricle also lasts longer

Fig. 15. × ⅔. Simultaneous record of pressures in left subclavian artery and in left ventricle. Read from right to left.

70 mm. ventricular manometer = 200 mm. Hg.
83 mm. subclavian manometer = 200 mm. Hg.[1]
Dog 8·5 kilos; heart 85 gms.

	T.	B.P.	V.P.	O.P.	Rate	Max. vent. press.	Time to raise pressure 60 mm. Hg.
a.	35·3° C.	78	32	228	22	109	0·050"
b.	35·3	156	40	228	22	198	0·086

---

[1] The calibration here and elsewhere refers to the original tracings, and not to the figures as reduced.

with the higher arterial resistance, and the change in diastolic tension seems to be conditioned more by the inadequate relaxation at the high pressure as compared with the relaxation at the low pressure. It must be borne in mind that the volume of the heart would be considerably bigger at the high pressure than at the low pressure.

II. THE REACTION OF THE HEART TO VARYING INFLOW.

Within wide limits the output of the heart is determined by and equal to the inflow into the right auricle. A heart of 50 gms. in good condition puts out as much blood as it receives, whether this be 200 or 2000 c.c. in the minute. We have investigated the changes in the volume of the ventricles and the changes in the pressure within the ventricles which accompany this reaction of the heart to alterations in venous inflow.

Changes in the volume of the heart. The effect of alterations in venous inflow on the systolic and diastolic volumes of the ventricles is shown in Fig. 16, where the cardiometer record taken on a slowly moving drum is compared with the arterial pressure and with the venous pressure. In the first part of this tracing the heart was beating regularly and put out 86 c.c. of blood every ten seconds against an arterial pressure of 124 mm. Hg. The venous inflow was now increased and the output of the heart, as measured on the other side of the arterial resistance, rose to 140 in 10 seconds. The outflow per beat, obtained by measuring the flow through the artificial arterial resistance, was 3·9 c.c. in the first case and 6·4 c.c. in the second case. A direct measurement of the cardiometer curves gave 5·75 c.c. and 8 c.c. respectively, but this would include also the amount of blood flowing through the coronary circulation, which at this high arterial pressure would be considerable. It will be seen that the heart adapts itself to the increased inflow in almost precisely the same fashion as it adapts itself to increased arterial resistance. The heart has naturally to set up a greater pressure in its cavities in order to drive out a larger amount of blood against the same arterial resistance as before. Theoretically one might imagine that the heart, being increased in size during diastole by the greater inflow of blood, might react so perfectly that the increased contractile stress set up as the result of the lengthening of the fibres would be sufficient to empty out the whole of the surplus blood it had received, so that the heart would go on beating with a larger excursion at each beat and a greater diastolic volume but with the same systolic volume as before. This condition however we have never met, though we would admit the possibility of its occurrence in a heart with high physiological efficiency. In every case we have found that the result of increasing the inflow is to increase the systolic as well as the diastolic volume, even when care is taken by adjustment of the artificial resistance to keep the mean pressure in the aorta constant. Even under this condition, although the pressure in the aorta at the end of diastole will be lower, the pressure at the end of systole will be higher than when the output per beat is smaller, and it seems probable that in order to keep

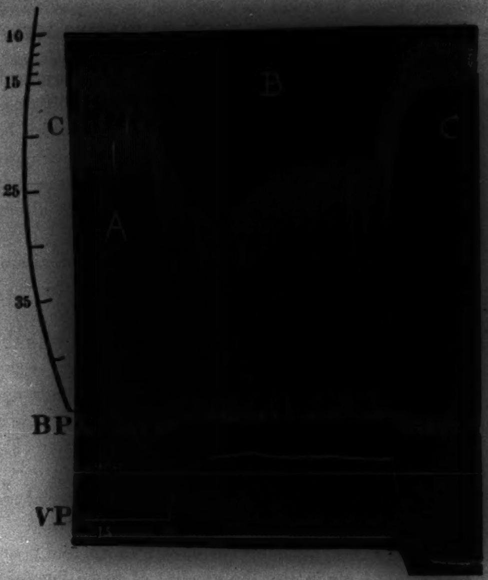


Fig. 16. Effect of alteration in venous supply on volume of heart. Read from left to right.

Dog 5·15 kilos; heart 67 gms.

	A.R.	B.P.	V.P.	Rate	O.P.	O.P. per beat calc.	O.P. per beat observed
A.	100	124	95	22	86	3·9	5·7
B.	100	130	145	22	140	6·4	8·0
C.	100	122	55	22	33	1·5	2·5

the systolic volume constant, we should have to diminish the arterial resistance to such an extent that the maximum systolic pressure in the aorta remained the same as before. We thus get with increased inflow increase in volume of the heart, which in the curve represented in Fig. 16 proceeds for 24 beats. We now get partial recovery of heart volume, the causation of which has already been discussed, so that finally the heart takes up a volume which oscillates between a constant systolic and constant diastolic volume, both of which are greater than the corresponding volumes with a smaller inflow. On now reducing again the inflow to 33 c.c. per 10 seconds a reversed change takes place. Each heart beat is smaller, and finally the heart settles down to a new state, in which each ventricle is putting out 2·5 c.c. of blood per beat and both systolic and diastolic volumes are smaller than they were at the beginning of the experiment with a larger inflow of 86 c.c. per 10 seconds. It will be seen that the pressure in the inferior vena cava rises and falls with the venous inflow. The significance of these changes has been discussed in a previous paper. We would here only observe that these changes in venous pressure must not be taken as necessarily implying corresponding increase or diminution in the diastolic pressure in the ventricles, *i.e.* in the initial tension in the ventricular cavities at the beginning of systole. A greater flow from the superior cava into the right ventricle must involve a greater head of pressure in the former, even if the pressure in the right ventricle is 0. Moreover the inflow into the veins from the reservoir is continuous. The inflow into the ventricle occurs only during the intervals between its contractions. Any lengthening induration of the contracted state, and corresponding shortening of diastole, will therefore raise the average pressure as measured in the inferior vena cava, even though the ventricles relax so quickly as to allow a practically free inflow of blood into them during the diastolic period. The initial tension on the ventricular fibres must therefore be determined directly before we can attempt to dissociate the influence of the two factors on the process of contraction.

The volume changes at each contraction with varying venous inflow. In Fig. 17 we give a series of cardiometer tracings taken at a fast rate of the drum under varying conditions of inflow, the arterial resistance being kept constant, so that increasing inflow caused a moderate increase in the mean arterial pressure. The temperature was maintained constant, so that the rate did not vary. In successive steps the inflow, as measured by the output from the peripheral side of the resistance, was increased from 40 c.c. to 250 c.c. in 10 seconds. The amplitude of the curves naturally increases with increasing output, and this greater amplitude of excursion must mean a greater average steepness both of the diastolic and systolic parts of the curve. It is evidently quite impossible to speak of a uniform shape of the curves or even of a part of the curve, as Henderson has done(4). Such a uniformity could only exist if the rate of the heart beat were accurately adjusted to the rate at which the blood is flowing into and being expelled from the heart. The systolic part of the curve with the lower inflow (up to 143 c.c. per 10 seconds) shows the division into three phases, which have already been discussed in describing the effect of change in arterial resistance. As the heart becomes fuller and its output at systole greater, these divisions disappear, and the descent of the curve, which signalises the expulsion of blood, becomes uniform. The diastolic part of the curve shows the rapid increase in volume due to the inflow of blood under the venous pressure at the root of the vena cava. This venous pressure varied from 20 mm. H_2O up to 210 mm. H_2O. In all cases the effect of the auricular systole is well marked on the diastolic upstroke. With the moderate inflow the effect of the auricular systole on the curve is probably exaggerated by the auricular muscles pulling on the ventricular wall and so causing some shifting of the elastic diaphragm closing the cardiometer. This on the curve would give an impression of a check in the rate at which the heart was being filled. With the high inflow and output of 238 and 250, the heart was reaching its limit of distensibility towards the end of diastole. This is shown by the fact

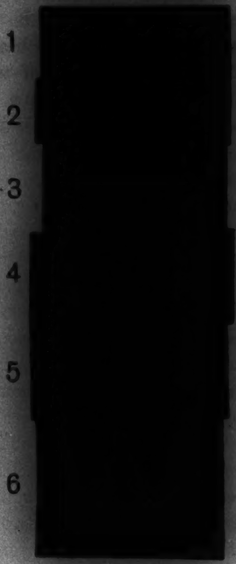


Fig. 17. Cardiometer tracings from the ventricles with varying inflow. Systole caused a movement downwards. Curves to be read from left to right. The tracings were taken under the following conditions.

Time marking = ⅕″.

	T.	B.P.	V.P.	O.P.
1.	34·4° C.	88	20	40
2.	—	88	65	111
3.	—	88	75	143
4.	—	100	115	196
5.	—	104	145	238
6.	—	110	210	250

that a rise of venous pressure from 145 to 210 only caused an increased output of 12 c.c. in the 10 seconds, whereas a rise from 75 to 115 mm. H_2O in curves 3 and 4 caused an increased output of 53 c.c. There is in consequence an almost flat top to the curve just before the auricular systole occurs. This flat top would correspond to the period of diastasis described by Henderson(4). As pointed out by Straub(18), it cannot be regarded as a normal feature in the volume curve of the ventricles but occurs only when the inflow during diastole, either in consequence of rapidity of inflow or slowing of the rate of the heart, is so great that

Fig. 18. × ¾. Simultaneous tracings of pressures in left ventricle and left auricle taken with varying venous inflow. Read from right to left.

Dog 10·5 kilos; heart 84 gms.

85 mm. ventricular manometer = 200 mm. Hg.

42 mm. auricular manometer = 200 mm. H_2O.

	T.	B.P.	V.P.	O.P.
a.	34·6° C.	100	25	338
b.	34·6	100	18	108

In *a* a rise of pressure of 80 mm. Hg. occupied ·041″; in *b* the same rise of pressure occupied ·061″.

the ventricle attains its limit of distensibility under the maximum venous pressure possible under the circumstances of the experiment before the onset of the next beat.

The effect of varying inflow on the pressure changes in the heart. If the mean arterial pressure is maintained constant while the inflow is increased, the output of the left ventricle is increased in proportion. The arterial resistance must therefore be diminished with increased inflow, and the arterial blood-pressure will show larger oscillations, the systolic pressure being increased, while the diastolic pressure is diminished.

In Fig. 18 we give curves of two observations taken one immediately after the other on the same paper without altering the position of the manometers. In the first record (*a*) the mean arterial pressure was 100 mm. Hg. and the output was 338 c.c. in 10 seconds—over two litres a minute. The heart was fairly large—84 gms.—and in excellent condition, as shown by the fact that the pressure in the inferior vena cava with this large inflow was only 25 mm. H_2O. The inflow was then reduced so that the output fell to 108 c.c. in 10 seconds, the arterial pressure being maintained at the same level as before, by increasing the arterial resistance. The pressure in the inferior vena cava fell to 13 mm. H_2O. The pressures were taken in the left ventricle and the left auricle. The first point we would draw attention to is the fact that the initial tension on the ventricular muscle at the commencement of contraction with the large output is the same or even a fraction lower than with the smaller output. It will be seen also that the auricular pressure, measured with a much more sensitive manometer directly after the auricular systole, falls to the same level when the heart has to expel 338 c.c. of blood per 10 secs. as when it has to expel only 108 c.c. per 10 secs. There is thus no difference in the initial tension on the muscle fibres of the ventricle to evoke any corresponding difference in the evolution of energy in the contracting muscle. But the difference in response of the ventricle in the two cases is marked. In the contraction with the larger inflow the rate of rise of pressure is more rapid and the maximum pressure produced is higher. In curve *a* the heart takes ·041 sec. to raise the pressure to 80 mm. Hg. In curve *b* the ventricle takes ·061 sec. to raise the pressure to the same extent. We do not observe here the great prolongation of the contracted condition which we noticed with the rise of arterial pressure, but this, as we have seen, is a question of the length of the muscle fibres, *i.e.* the volume of the heart, during the continuance of the contracted state.

Fig. 19 shows still more strikingly the absence of relation between the energy of the ventricular contraction and the initial tension on its muscle fibres. It has been already pointed out in a previous paper that with increasing venous inflow there is at first hardly any rise of pressure in the inferior vena cava. It only becomes marked as the heart is approaching its limits of distensibility. So long as the pressure in the inferior vena cava remains low, diastolic pressure in the ventricle will depend essentially on the rate of relaxation of the ventricular muscle rather than on slight variations in the pressure in the inferior vena cava. A slight change in the physiological condition of the heart muscle, which might alter this rate of relaxation, might therefore have a considerable influence on the actual diastolic pressure attained in the ventricle.

In the experiment from which curves in Fig. 19 were taken the heart had been fed for some time at an arterial pressure of only 75 mm. Hg. The pressure was then put up to 116, with an output of 102 c.c. per 10 secs., and the first curve was taken. The output was then diminished to 50 c.c. per 10 secs. It will be noticed that the diastolic pressure with the lower output is actually slightly higher than with the larger output. The inferior vena cava pressure in the two cases was 36 and 14 mm. H_2O, so that the two tracings were taken within the limits at which the rate of relaxation of the heart is the determining factor for the diastolic pressure in the ventricle.

Fig. 19. × ¾. Simultaneous tracings of pressure in the left subclavian stump and in left ventricle with varying venous filling. Read from right to left.

Dog 8·5 kilos; heart 85 gms.

70 mm. ventricular manometer=200 mm. Hg.

	T.	B.P.	V.P.	O.P.	Rate
a.	34·4° C.	116	36	102	20
b.	34·4	116	14	50	20·5

As soon as the alterations in venous inflow are attended with marked alterations in venous pressure, we observe a similar change in the diastolic pressure in the ventricles. Towards the end of an experiment, when the heart is dilating, any slight alteration in inflow causes a corresponding change in the inferior vena cava pressure and in the diastolic pressure in the ventricles. In Fig. 20 we give two curves taken at different inflows and showing the pressure in the left ventricle and the volume of the two ventricles. The changes in the ventricular curve are similar to those described in Fig. 18, but for the fact that the diastolic pressure immediately before the commencement of systole is slightly higher in the curve with a larger inflow. In these cases the output varied between 55 c.c. and 180 c.c. in the 10 seconds. The pressure in the inferior vena cava varied between 50 and 106 mm. H_2O. A still bigger effect is shown in Fig. 21, where in a small heart of 49 gms. the output was altered from 172 to 54 c.c. in 10 secs., with a corresponding fall in venous pressure from 100 to 22 mm. H_2O. In the curves represented in Figs. 20 and 21, the cardiometer tracings show the changes which we have found to occur invariably with alterations in venous inflow. We thus find that in the reaction of the heart to increased venous inflow, as in its reaction to increased arterial resistance, the only

Fig. 20. $\times \frac{2}{3}$. Simultaneous tracings of pressure in left ventricle and volume of the two ventricles, with varying inflow. Read from right to left.

Dog 6·5 kilos, heart 70 gms.

54 mm. ventricular manometer = 200 mm. Hg.

	T.	B.P.	V.P.	O.P.	Rate
a.	36·2° C.	96	50	55	25
b.	36·7	94	106	180	25·5

factor which constantly varies with the response of the ventricles is the volume of the heart, *i.e.* the length of its muscle fibres, and we are therefore justified in ascribing the increased energy of the heart's contraction under conditions of increased pressure or increased inflow to alterations in the length of the muscle fibres at the beginning of and during their state of excitation, and not to changes in the tension on the fibres, which, as we have seen, may or may not be present at the same time.

Fig. 21. As Fig. 20. Dog 9·5 kilos (pregnant); heart 49 gms.

	T.	B.P.	V.P.	O.P.	Rate
a.	36·2° C.	90	100	172	20·5
b.	35·8	90	22	54	19·5

III. THE INFLUENCE OF RATE.

In our preparation the rhythm of the heart is determined entirely by the local condition of the heart muscle and is a function simply of the temperature of the pace maker. Its average rate at the temperature of the body, 37° C., is about 160 per minute as compared with the normal rate in the intact animal of 70 to 90 per minute. We can reduce its rate by stimulation of the vagus nerves or by cooling the blood with which it is supplied, or by administration of carbon dioxide by the lungs. We can increase its rate by raising the temperature, by stimulation of its sympathetic supply, or by administration of adrenalin. We propose here to deal with the influence of two of these factors, namely stimulation of the vagus and cooling, both of which will diminish its natural rate.

Of the two the influence of the vagus may be regarded as having the more significance, since it is by the influence of the tonic impulses descending this nerve that the heart rate in the intact animal is kept at its normal level.

The influence of the vagus. The general effect of stimulation of the vagus on the volume of the heart is well known. As the heart slows, it becomes larger owing to the greater time allowed for the inflow of blood. When it contracts it therefore puts out more blood. The slowing of the heart leads to a lowering of the diastolic pressure. In the heart lung preparation the diastolic pressure is set by the arterial resistance. Below this point the blood-pressure can only fall by the leakage of blood from the arterial side of the system through the coronary vessels. The systolic pressure will therefore be greater when the heart is slowed than when beating at its normal rate, since the output of blood at each beat is increased.

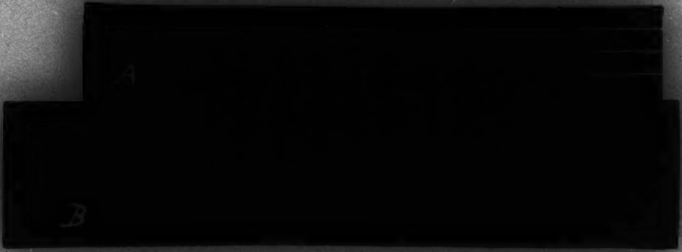


Fig. 22. Cardiometer tracings of dog's heart in heart lung preparation. Read from left to right. Systole caused a movement of the lever downwards.

	T.	B.P.	V.P.	O.P.	Rate
a. Normal tracing	35° C.	80	75	124	26
b. Left vagus stimulated	35	?	200—220	106	12·5

To the right of *a* is placed the calibration of the cardiometer, the space between two horizontal lines corresponding to an output of 5 c.c.

The effect of vagus slowing and the variations in cardiac volume accompanying each beat are shown in Fig. 22. In this experiment, by stimulation of the left vagus, the heart was slowed from a rate of 26 in 10 secs. to one of 12·5 in 10 secs., the conditions of inflow remaining constant. The output fell from 124 c.c. to 106 c.c. in 10 secs., and there was therefore a considerable rise in the pressure in the inferior cava, namely from 75 to 220 mm. H_2O. At this point the distensibility of the heart, or at any rate of its right side, must have been approaching its limits, and we therefore find that the diastolic part of the curve shows the flat top towards the end, the causation of which we have already discussed. (The small elevations on the curve are due to the contractions of the auricles which under the influence of the vagus were beating independently of the ventricles.) The duration of the contraction was approximately the same under vagus stimulation as it had been before, the slowing being dependent on a prolongation of the diastolic period or period of filling.

It is evident that under such conditions as those under which Fig. 22 was taken there must be a considerable rise of diastolic pressure in the right ventricle, and probably also in the left ventricle, though it must be remembered that the diastolic supply of blood to the left ventricle depends on the contraction of the right ventricle and is not unlimited, as is the case on the right side of the heart which is connected directly with the inexhaustible venous reservoir. Such a rise of diastolic pressure is given in the curves in Fig. 23, where the pressure in the left ventricle is taken first with stimulation of the left vagus, which gave rise to extreme slowing, and secondly when the heart is beating freely. The pressure in the inferior vena cava rose from 60 to 220 mm. H_2O, and it will be seen that when the ventricle starts

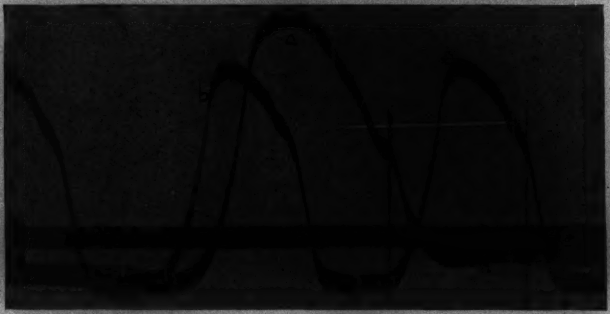


Fig. 23. × ⅔. Pressure curve from left ventricle. Read from right to left.
Dog 8·5 kilos; heart 66 gms.
88 mm. ventricular manometer = 200 mm. Hg.

		T.	B.P.	V.P.	O.P.
a.	Left vagus stimulated	35° C.	?	220	?
b.	Normal tracing	35	90	60	100

to give its single beat in curve *a*, the pressure in the left ventricle is 7 mm. above the base line, corresponding to a pressure of about 16 mm. Hg. After the beat the pressure sinks to the zero line and then gradually rises again. Since, as we know, the volume of the heart must have been considerably greater under vagus stimulation than when beating at its normal rhythm, we might expect the rise of pressure in the ventricles to be more rapid under vagus stimulation than with the normal beat. As a matter of fact the reverse is the case. The rise of pressure is somewhat slower and the rise of the curve after the opening of the aortic valves is no more rapid than in the normal tracing, although the maximum height to which the pressure rises is considerably greater.

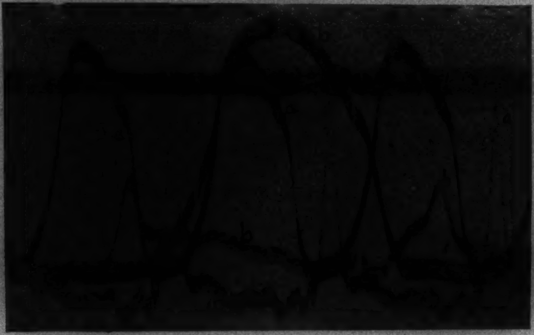


Fig. 24. × ¾. Simultaneous pressure tracings taken in the left ventricle and in the left auricle. Dog 10·5 kilos; heart 84 gms.
85 mm. ventricular manometer = 200 mm. Hg.
42 mm. auricular manometer 200 mm. H_2O.

	T.	B.P.	V.P.	O.P.
a. Normal tracing	35° C.	100	16	139
b. Left vagus stimulated	35	100	50—60	?

The prolongation of the duration of contraction seems to indicate that length of fibre is still effective in increasing the amount of energy set free during the contraction, and the duration of the excitatory state, but the slow rate of rise of the pressure shows that the effect of the vagus is not confined to altering the rhythm, as determined by the pace maker, but is a direct one on the processes of the ventricular muscle itself. The process of relaxation is seen to occur at the same rate as in the normal contraction.

In Fig. 24 we have a similar tracing, in which the pressure in the left auricles is also measured. Here the pressure in the left ventricle comes down to the base line and only rises slightly above it as the effect of auricular systole. In this case the pressure in the inferior vena cava only rose from 16 to 50 mm. H_2O during stimulation of the vagus. The process of relaxation is somewhat slower in the vagus beat than in the normal beat. The vagus would thus seem here to slow all the processes occurring in the ventricular muscle though not to abolish the fundamental relation between length of muscle fibre and amount of energy evoked during contraction.

In the experiment from which Fig. 25 was taken the effect of the vagus was to produce a more moderate slowing of the heart, namely,

Fig. 25. $\times \frac{3}{4}$. Simultaneous pressure tracings in left ventricle and in subclavian artery. Read from right to left. (Note. The mirror on the subclavian manometer was obscured, so that the tracings from this are hazy.)

Dog 8·5 kilos; heart 85 gms.

70 mm. ventricular manometer = 200 mm. Hg.

	T.	B.P.	V.P.	O.P.	Rate	Max. press.
a. Left vagus stim.	36° C.	114	50—140	?	16·5	150
b. Normal tracing	36	114	28	189	24	184

Rise of pressure of 90 mm. Hg. in *a* took ·046″ and in *b* ·040″.

from 24 to 16·5 in ten seconds. The pressure in the veins entering the right side of the heart rose from 28 to a height varying between 50 and 140 mm. H_2O. In this experiment we were recording also the pressure in the aorta, and the larger rise of pressure accompanying the vagus beats was evidence of the greater outflow from the left ventricle at each beat and therefore of the greater filling of these cavities. In spite of this greater filling it will be seen that the diastolic pressure under vagus stimulation falls to a lower level than during the normal beat. The rate of rise of pressure was only slightly slowed, but the rate of fall of pressure was somewhat more definitely slowed. The effect of the vagus on the ventricle is therefore to prolong diastole without a corresponding rise in diastolic pressure, so that the inflow of blood into the ventricles is facilitated. It slows the rate of rise of pressure but still allows the heart to respond to its filling by increased duration of contraction, and thus produces a contraction which seems more adapted to driving the mass of viscous blood into the aorta against resistance than the short sharp beat of the unbridled heart of the heart lung preparation.

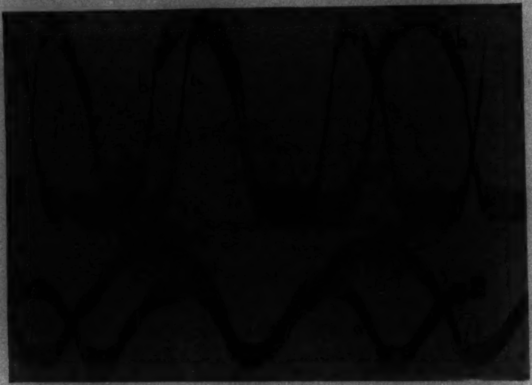


Fig. 26. Simultaneous records of pressure in left ventricle and volume of both ventricles. Read from right to left.

Dog 6·5 kilos; heart 70 gms.

	T.	B.P.	V.P.	O.P.	Rate	Max. press. mm. Hg.
a.	36·8° C.	100	104	153	25·5	150
b.	31	100	114	158	16·5	160

A study of the pressure conditions ruling in the heart during the different phases of the heart's cycle tend therefore to support the views which are commonly held as to the sparing influence of the vagus on the heart.

The influence of temperature. It has been shown (Markwalder and Starling (17)) that alteration of the rate of the heart by changes of temperature, keeping the venous inflow constant, causes no alteration in the total output of the heart, though the falling rate induced by diminution of temperature is attended by a rise of venous pressure as measured in the inferior vena cava. Thus in one experiment with a rate of heart beat of 156 per minute at 39° C., the outflow was 650 c.c. and the venous pressure was 38 mm. H_2O; with a rate of 72 and a temperature of 28°·2 C. the output was 652 c.c. per minute and the venous pressure 110 mm. H_2O. On inspection, the heart, as it cools, though filling more at each diastole, seems to empty itself as effectively, or rather more so, at the lower as at the higher temperature. The influence of a moderate fall of temperature, namely from about 37° to 31° C., and from 34°·3 to 30° C. is shown in Figs. 26 and 27. In Fig. 26 the pressure in the left ventricle was taken simultaneously with the record of the volume of the two ventricles. The rate of beat dropped from 25·5 in ten seconds to 16·5 in ten seconds. Both records gave evidence of an improvement in the physiological state of the heart muscle, which we have taken to be synonymous with tone. These cardiometer curves show that whereas the diastolic volume was equal in the two cases, the systolic volume is diminished proportionately to the diminution in the number of heart beats, so that the heart empties itself more completely at each contraction. With this moderate difference of rate there was only a slight increase in the venous pressure in the vessels entering the right side of the heart, namely from 104 to 114 mm. H_2O. The output remains steady at 153 c.c. per ten seconds. The diastolic pressure in the left ventricle is seen to be identical at both rates. The rate of rise as well as the rate of fall of pressure is slightly greater in the cooled than in the warm heart. The main difference between the two conditions is the prolongation of the excitatory state and the prolongation of the diastolic state. In consequence of this prolongation of the process of contraction the heart is able to send out the increased amount of blood which has entered it during diastole with hardly any increase in its maximum pressure.

A similar story is told by the curves given in Fig. 27, taken from an experiment in which the aortic pressure was registered simultaneously with the pressure in the left ventricle. Here we have diastolic pressure identical at the two temperatures, the rate of rise and the rate of fall of pressure also practically identical, the only change being in the duration of the contracted state and of the relaxed state. If the cooling of the heart is carried further to 28° or even 26° C., this slowing of the processes operative in the heart muscle increases to such a degree that both rise and fall of the pressure curve are markedly slowed. Within the limits employed in the two experiments recorded, it would seem that the effect of lowering the temperature is purely beneficial in economising the energies of the heart muscle. The same conclusion may be drawn from Evans' observations on the efficiency of the heart muscle and the relation of its total metabolism to the mechanical work which it performs at various temperatures. It must be remembered however that the mammalian heart is not merely able to carry out a constant amount of work but is always ready to respond in moments of stress or danger to an enormous increase in the demands made upon it. We have already seen (Patterson and Starling (6)) that the maximum amount of blood which can be put out by the heart is a function of its rate. Moreover a cooled heart cannot respond to the action of adrenalin in the manner that a heart will at the normal body temperature. In the intact animal therefore, the sparing effect, which in the heart lung preparation we can produce by cooling the organ, is produced by the constant tonic influence exercised by the vagus centre.

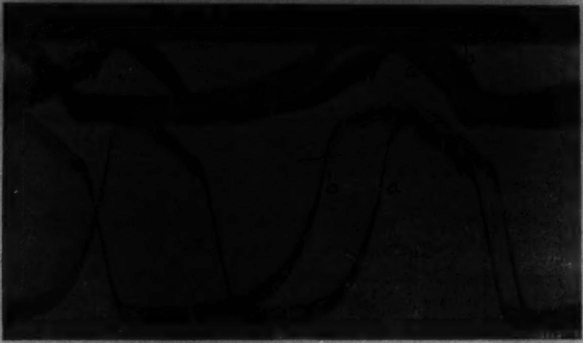


Fig. 27. Simultaneous records of pressure in left ventricle and in subclavian artery, to show influence of cooling. (Owing to parallax the arterial record is shifted about 1·5 mm. to the left of the ventricular record.) Read from right to left.

	T.	B.P.	V.P.	O.P.	Rate	Max. press. mm. Hg.
a.	34·3° C.	120	20	109	21	120
b.	30	120	26	109	14	132

Finally it should be mentioned that the cooled heart differs in no respect from the warm heart in its reaction to changes in venous inflow or changes in arterial pressure. Every increase in either of these factors gives an increased diastolic volume of the heart and increased mechanical response at contraction. With a moderate increase in inflow or arterial resistance, the diastolic pressure in the ventricles may be unaltered. If the increase in size during diastole becomes excessive, such an increase can only be produced by an active stretching of the ventricular wall, and we then find that the diastolic pressure, and therefore the initial tension on the muscle fibres at the commencement of contraction, is increased at the same time as the cardiac volume. In every case therefore the reaction of the heart muscle seems to be determined by the length of its constituent fibres at the moment of activity. Changes in initial tension are merely incidental to changes in length.

REFERENCES.

(1) **Blix.** Skand. Arch. f. Physiol. III. p. 295. 1892; IV. p. 399. 1893; V. pp. 150 and 173. 1895; VI. p. 240. 1895.
(2) **Frank.** Ergebn. d. Physiol. III. II. p. 348. 1904.
(3) **A. V. Hill.** This Journal, XLVI.
(4) **Yandell Henderson.** Amer. Journ. Physiol. XVI. p. 325. 1906.
(5) **Patterson and Starling.** This Journal.
(6) **Frank.** Ztsch. f. Biol. XXXVII. p. 516. 1899.
(7) **Frank.** Ztsch. f. Biol. XXXII. p. 370. 1895.
(8) **Rohde.** Arch. f. Exp. Path. u. Pharm. LXVIII. p. 401. 1912.
(9) **Hermann Straub.** Deutsch. Archiv f. Klin. Med. CXV. 531. 1914.
(10) **Hermann Straub.** This Journal, XL. p. 378. 1910.
(11) **Heer.** Pflüger's Arch. CXLVIII. p. 1. 1912.
(12) **Piper.** Arch. f. (Anat. u.) Physiol. p. 343. 1912.
(13) **Hermann Straub.** Pflüger's Arch. CXLIII. p. 69. 1911.
(14) **Markwalder and Starling.** This Journal, XLVII. p. 275. 1913.
(15) **v. Anrep.** This Journal, XLV. p. 307. 1912.
(16) Quoted by **Hürthle.** Pflüger's Arch. XLIX. p. 74. 1891.
(17) **Markwalder and Starling.** This Journal, XLVIII. p. 348. 1914.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

January 24, 1914.

Note on the hypodynamic heart. By W. BURRIDGE.

The reaction to a tetanising current of the fresh heart of the frog beating on and circulating its own blood is that of a 'potassium' heart.

This reaction is changed when Ringer's solution is perfused through the heart.

Ringer's solution as usually made up contains an excessive quantity of calcium (0·012 °/₀–0·025 °/₀ $CaCl_2$).

The extra calcium combines with and removes potassium phosphate from the heart.

There is little or no failure of contraction during the first 24 hours in hearts perfused with saline mixtures: there is failure of the excitation process.

The irregularities etc. wrought during the first 24 hours in the frog's heart by perfusing it with the usual inorganic saline mixtures may apparently be completely removed by treatment with potassium phosphate.

By replacing the phosphate at intervals the frog's heart may be made to beat up to the 4th day after commencement of perfusion.

Treatment with potassium phosphate may restore rhythm to the heart 'exhausted' and rendered quiescent by long continued perfusion of the usual inorganic salt mixtures.

Adrenalin may benefit the hypodynamic heart.

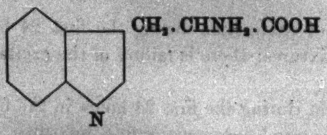
A note on the excretion of kynurenic and uric acids by dogs. By Annie Homer, *Beit Memorial Fellow.*

It is generally accepted that dogs do not excrete uric acid to any appreciable extent. The results of some of my feeding experiments go to prove the contrary.

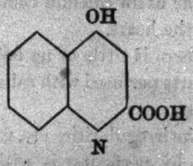
Liebig (*Annalen*, LXXXVI. p. 125. 1853) first showed that dogs on a meat diet excrete kynurenic acid and Ellinger (*Zeit. Phys. Chem.* XLII. p. 325) has demonstrated that the output of this substance is markedly increased by the administration of tryptophane.

A series of feeding experiments extending over a period of two years has been undertaken in order to ascertain whether the formation and elimination of kynurenic acid represented the main line of the breakdown of tryptophane in the metabolic processes of the dog. The results of these experiments indicate that the amount of kynurenic acid excreted in the urine does not bear any simple relationship to the amount of tryptophane administered. It was found that the age and size of the dog had to be taken into consideration, and from a study of the analytical results it was obvious that the formation of kynurenic acid did not give a complete picture of the fate of tryptophane in the organism.

Taking into consideration the results obtained above and bearing in mind that the direct formation of kynurenic acid from tryptophane by the animal body must involve the preliminary disruption of the pyrrol ring of an indol nucleus and the introduction therein of an extra carbon atom to form a quinoline compound



Tryptophane (Ellinger)



Kynurenic Acid (Homer),

it is conceivable that kynurenic acid has not been formed primarily from tryptophane itself but rather as the result of more obscure metabolic processes which have been induced by the administration of tryptophane or of precursors of tryptophane.

In connection with these experiments I found that the kynurenic acid estimated (Capaldi's method) was contaminated with a substance which proved to be uric acid.

In a further series of experiments I ascertained that the amount of uric acid and of kynurenic acid excreted depended on the diet given to the dog. On rations of bread and milk traces only of these acids were excreted whereas on those rich in meat appreciable amounts of uric acid as well as of kynurenic acid were eliminated in the urine. Thus on a meat free diet two dogs, *A* of 13½ lbs. and *B* of 23½ lbs. weight, excreted traces only of uric acid whereas on 500 grs. meat per diem they excreted as much as 0·2 and 0·3 grs. uric acid respectively in a period of 24 hours.

My results show that in the estimation of kynurenic acid by Capaldi's method unless the dogs are kept on a meat free diet the substance finally weighed is a mixture of kynurenic and uric acids and cannot be taken as a true representation of the kynurenic acid output.

I have also shown that the presence of kynurenic acid does not in any way interfere with the estimation of uric acid whether by Hopkins' method or by a modification of his method employed by me.

I hope shortly to publish the data in full.

The occurrence in ergot and action of acetyl-choline. By H. H. DALE. (*Preliminary communication.*)

The intense depressor activity of acetyl-choline was described by Hunt and Taveau, who examined a series of choline-esters, and found that acetyl-choline lowered the blood-pressure in doses far smaller than the minimal pressor dose of adrenine. I have been familiar for some time with a pronounced inhibitor effect on the heart, shown by some specimens of ergot, and always associated with an intense stimulant action on intestinal muscle. Both actions were abolished by atropine. Since none of the known principles accounted for this action, the chemical nature of the substance responsible for it was investigated by A. J. Ewins, who was able to isolate it and identify it as acetyl-choline.

I find that acetyl-choline in minute intravenous doses (0·0001 mgm. to 0·001 mgm.) produces a vasodilator fall of blood-pressure without significant effect on the heart. It dilates the vessels of the perfused rabbit's ear. In somewhat larger doses (0·01 to 1 mgm.) it causes also pronounced vagus-like inhibition of the heart, and various other effects of stimulating nerves of the cranial and sacral divisions of the autonomic system—secretion of saliva, contraction of the œsophagus, stomach and intestine, and of the urinary bladder. The effects, though intense while they last, are remarkably evanescent, and are repeated with striking uniformity if a series of similar injections is given. Presumably the ester is rapidly hydrolysed in the circulation into its relatively inert components. The action on plain muscle which is innervated only by nerves of the true sympathetic system, such as that of the uterus, is relatively slight. The pupil forms an exception among structures innervated by cranial autonomic nerves, constriction being absent or insignificant.

The action is best seen on isolated organs, such as a loop of intestine, or the perfused heart of the frog. The latter shows distinct inhibition with a dilution of 1 in 1,000 millions.

Hypodermic injection of larger doses (20 mgms.) in the cat produces promptly the picture of intense cranio-sacral autonomic stimulation—salivation, lachrymation, slow heart-beat, defæcation, erection of the penis. Some secretion of sweat was also observed. Atropine in small doses abolishes the effects of acetyl-choline, but the antagonistic effect is less powerful than in the case of muscarine.

A note on a new method of estimating tryptophane in proteins. By Annie Homer, *Beit Memorial Fellow.*

Tryptophane cannot be isolated in any appreciable quantity from the products formed during an acid hydrolysis of proteins. I have shown that 25 per cent. sulphuric acid has a very slow action on pure tryptophane but the presence of an oxygen carrier accelerates the action of the acid.

It is conceivable that during the acid hydrolysis some decomposition product of the protein molecule acts as an oxygen carrier or that other amino acids serve as catalysers and induce the decomposition of tryptophane as quickly as it is split off from the complex molecule of the protein.

I have ascertained that when baryta acts on protein the tryptophane liberated during the hydrolysis is not destroyed. This observation has been made the basis of a method for the estimation of the tryptophane content of proteins.

The protein is hydrolysed with baryta and the excess of baryta removed with sulphuric acid. The tryptophane is precipitated in the usual way with mercury sulphate and washed free from any tyrosine. The mercury sulphate compound is decomposed with sulphuretted hydrogen. The filtrate from the precipitate of mercury sulphide is freed from sulphuretted hydrogen and sulphuric acid, and the tryptophane content is estimated against both nascent and molecular bromine which have been standardised against a known solution of pure tryptophane.

Three different samples of commercial casein have been hydrolysed in this way. The results for separate determinations of the same samples have shown remarkable concordance; further each sample gave values from 1 to 1·5 grs. of tryptophane per cent. of casein and these numbers are in very fair agreement with those obtained by Hopkins and by Abderhalden (1·5 per cent.) for pure casein.

I do not pretend to assume that the values obtained above represent the whole amount of tryptophane in the protein, but it can be taken that they afford minimal values.

I am applying the method to the estimation of the tryptophane content of other proteins; even minimal values will be of more service in nutrition work than the present way of expressing the tryptophane content of the hydrolytic products of proteins, viz. merely as + *ve* or − *ve*.

The homonymous induction of colour. By F. W. EDRIDGE-GREEN.

Demonstration.

(*From the Institute of Physiology, University College, London.*)

If a black surface from which no light is reflected be surrounded by spectral light of any colour the black surface will appear of the same colour as that surrounding it and steadily increasing in luminosity. In no case is there the least appearance of the complementary colour unless the eye be slightly moved, and then it is seen on the black surface. This experiment can be made equally well with a deep red glass which does not transmit any yellow or green rays. If on a sheet of such red glass an opaque black patch an inch square be pasted and this be held up to the light and intently regarded with one eye the black surface will appear uniformly red of steadily increasing luminosity. The complementary blue-green will only appear when the eye is moved.

The action of muscarine on the electrical response of the frog's heart. By G. R. MINES. (*Preliminary note.*)

Muscarine hydrochloride (synthetic) in concentrations ·001 °/₀ to ·005 °/₀ in Ringer's solution ($C_H \cdot 10^{-8}$) perfused through the frog's heart reduces the frequency of the automatic beats[1]. Apart from this action it greatly reduces the duration of the electric disturbance in the ventricle: closely related to this effect is the reduction in force of contractions which the drug produces. The A.V. interval is little if at all affected. The various effects of muscarine are all antagonised by atropine; the effect on the frequency of the beat may be eliminated by the carefully localised application of atropine to the sinus, without preventing the action of the drug on the ventricle.

The effects on the electrogram produced by local application of muscarine solutions to the surface of the heart depend essentially on the property of the drug of altering the duration of the electric disturbance in the muscle. Straub's experiments on the ventricle are in accordance with this interpretation[2].

Application of muscarine to the sinus venosus, if strictly localised to this region, produces no effect on the ventricular electrogram other than that due to simple slowing. Samojloff's conflicting statement on this point must be ascribed to a spread of the drug from the sinus to the base of the ventricle in his experiments[3]. The application of a trace of muscarine to the base of the ventricle causes a change in the form of the electrogram in which the final variation becomes negative in sign. Conversely, application to the apex of the ventricle causes the final variation to become positive. (The galvanometer leads are in every case from the apex of the ventricle and the base of the heart. Under these circumstances, as in the ordinary electrocardiogram, the part of the curve due to the auricle (P-wave) and the initial variation of the ventricular complex (R-wave) are both in the direction spoken of as "positive.")

In a heart perfused with a solution devoid of calcium, in which inhibition of the electrical responses can no longer be produced by electrical stimulation of the vagus, whether pre- or post-ganglionic, muscarine produces its usual effects on the electric responses, and these effects are antagonised by atropine.

Natural muscarine acts qualitatively in the same way as synthetic muscarine. The action of arecoline is closely similar.

---

[1] For details of method see *Journ. Physiol.* XLVI. p. 189. 1913.

[2] *Zentrlbl. f. Physiol.* XXVI. p. 990. 1912.

[3] *Ibid.* XXVII. p. 7. 1913.

A combined tonometer and electrode cell for measuring the H ion concentration of reduced blood at a given tension of CO_2.

By R. A. Peters. (*Preliminary communication.*)

The difficulty of obtaining quick and reliable determinations of the H ion concentration of blood has been shown by Hasselbach[1] and Konikoff[2] to be due to the presence of oxygen. In fact a true equilibrium does not seem to be obtained until the blood in contact with the electrode is completely reduced. Hence it comes about that these and other observers have been measuring in reality the H ion concentration of reduced blood. In the following method the blood is first reduced in an atmosphere of H_2, and then revolved in a tonometer against required tensions of CO_2. The O_2 is removed by means of evacuating and washing out with H_2 at a temperature of 37° C. Höber[3] and others have shown that CO_2 does not set up its own potential with the H electrode.

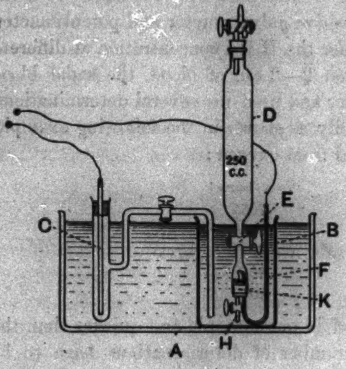


Fig. 1.

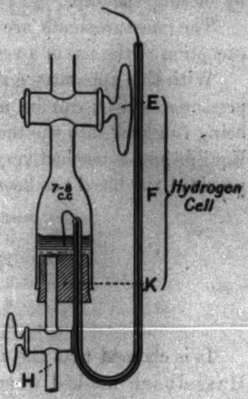


Fig. 2.

Figs. 1 and 2. *A*, wall of water thermostat. *B*, glass vessel filled with saturated potassium chloride. *C*, calomel half electrode. *D*, tonometer filled with H_2 or H_2+CO_2 mixture. *E*, glass tap. *F*, platinum wire blacked and saturated with H_2. *H*, glass tube with tap. *K*, rubber cork.

---

[1] *Biochem. Zeitschrift*, XLIX. 449. 1913.

[2] *Ibid.* LI. 202. 1913.

[3] Höber. *Arch. f. d. ges. Phys.* XCIX. 572. 1903.

The apparatus for the purpose is a tonometer to which is attached at one end a small glass cell containing a rubber cork through which pass the platinum H_2 electrode inverted as in diagram and a short piece of glass tubing containing a small tap through which connection is made with the saturated KCl solution and the calomel electrode by turning this tap completely round. The fluid inside the tap is found to give sufficient electrical connection. The saturated KCl acts also as a bath in which the H_2 cell part of the tonometer is immersed[1]. The whole is in a water thermostat. The blood is revolved in the main body of the tonometer for ten minutes until equilibrium is reached and then allowed to flow into the cell, where it is shut off from the tonometer by closing the tap.

The measurements are made as described by Sörensen[2] with the exception of the use of a very sensitive galvanometer and potentiometer.

With this apparatus a curve for the H ion concentration at different pressures of CO_2 can be made on 2—3 c.c. of blood, the same blood being run back into the tonometer and used for several determinations. Equilibrium is reached very rapidly as shown in the following example, provided the blood has been freed from oxygen:

Minutes	Volts observed
0	·7055
1½	·7056
3	·7057
6	·7058
9	·7058

It is claimed that the method is more simple and quicker than the Hasselbach method where a number of determinations have to be made at known pressures of CO_2, or at an alveolar pressure in blood.

It does not give the H ion concentration of oxyhæmoglobin, but that will not be possible until some other satisfactory electrode can be found.

N.B. Care must be taken in the evacuation to prevent the drying of the blood.

---

[1] This is the principle employed by Walpole, *Biochemical Journal*, VII. 1913.

[2] Sörensen. *Ergebnisse*, p. 398. 1912.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

February 14, 1914.

The influence of sodium chloride on the frog's heart. By W. BURRIDGE. (*Preliminary communication.*)

Sodium chloride perfused through the frog's heart in excess, *i.e.* in quantities above normal, tends to displace calcium salts from the sarcoplasm and from semi-permeable membranes. Whether actual displacement occurs or not depends on the relative concentrations of the sodium and calcium salts in the perfusing solution. Calcium salts can antagonise this action of sodium chloride, this being apparently the phenomenon investigated by Ringer. During this process the refractory period is lengthened.

Following on this primary change there is an alteration in the condition of the heart colloids such that the effective action of a given concentration of calcium salts is greatly increased. The refractory period is now greatly shortened and may be of no greater duration than $\frac{1}{8}$ of systole. The contractions are capable of summation and the ventricle may apparently be completely tetanised. Under certain conditions "veratria" contractions may be obtained. All these actions depend on an increased effectiveness of calcium.

On again perfusing "normal" saline there is a temporary exaggeration of the conditions just mentioned. This is followed by a change in the state of the colloids such that the effective action of calcium is much less than during the perfusion of the hypertonic solution. They do not return, however, to the original "normal" condition but greatly overshoot this point. Following on this there is a return swing back again to the original "normal" point. The "return swing" is a very slow developing change.

The immediate changes described by **Carlson** as occurring on perfusion of hypotonic saline solutions depend on the lessened restraint now imposed on the calcium. The refractory period is shortened during these processes.

Succeeding these events there are changes which result in a greatly decreased effectiveness on the part of calcium salts. The refractory period lengthens, etc.

On returning from this condition to "normal saline" there is a temporary exacerbation of the condition just mentioned, followed by a very slow return to the "normal," or original condition. The sequence of events noted on return from hypotonic sodium chloride to "normal" reproduce in a minor degree those observed on passing from "normal" sodium chloride to excess.

It would follow from these results that if the amount of sodium chloride in a perfusing solution be increased, the effective action of the calcium salts already present in the solution will also be increased but at a much later period and *vice versa*.

The oxidative removal of lactic acid. By A. V. Hill.

Lactic acid produced in a muscle by activity is removed under the influence of oxygen, but it has long been debated whether this is carried out (*a*) by a process of oxidation or (*b*) by a process of replacement into its previous position. **Fletcher** and **Hopkins** showed that the same amount of lactic acid is produced in a muscle either (*a*) by rigor direct or (*b*) by stimulation, oxidative recovery and final rigor: which fact rendered very probable, although it did not finally prove, the hypothesis of replacement. The following considerations seem to me to make the hypothesis of replacement almost certain. **Peters** (*Journ. of Physiol.* XLVII. p. 270. 1913) has shown that the production (practically under anaerobic conditions) of about ·2 % of lactic acid by stimulation leads to the evolution of about 0·9 calorie per grm. of muscle substance. The production of 1 grm. of lactic acid is accompanied therefore by the evolution of about 450 calories. Now I have shown (*Journ. of Physiol.* XLVI. p. 43. 1913) that during the recovery processes of muscles in oxygen there is a "recovery heat-production" of about the same order of size as the heat-production occurring in the initial processes of contraction. In the oxidative removal of 1 grm. of lactic acid therefore there is a heat-production of about 450 calories. Now the oxidation of 1 grm. of lactic acid leads to a heat-production of about 3700 calories, which is about eight times as large as the quantity observed. By no stretch of the imagination can the recovery heat-production in my experiments be regarded as being eight times as large as the initial heat-production; such an enormous delayed heat-production could not fail to be detected. Therefore apparently the lactic acid is not oxidised but replaced in its previous position, under the influence and with the energy of the oxidation, either (*a*) of a small part of the lactic acid itself, or (*b*) of some other body. Evidence given elsewhere shows that it must be of some other body. The lactic acid therefore is part of the machine and not part of the fuel.

The total energy available in isolated muscles kept in oxygen. By A. V. HILL.

In a recent paper I showed how the ratio of the heat-production to the tension developed by a muscle undergoing an isometric twitch, can be estimated in absolute units (*Journ. of Physiol.* XLVI. p. 462. 1913). Using sartorius muscles under low initial tensions and moderate stimuli, the heat-production, in 39 observations on five different muscles, appeared to be on the average about 5×10^{-6} calories per grm. wt. of tension developed per cm. of muscle length: the average divergence from this value was only 14 % of it, so that one may conclude that the quantity—5 microcalories—is relatively exact. It concerns of course only the "initial heat-production," or the heat set free immediately on excitation in the absence of sufficient oxygen: counting the recovery heat-production, for muscles with abundant oxygen, the value must be about 10 microcalories.

Now it is obvious that by using this figure we can estimate with some accuracy the heat-production of a frog's sartorius, under various conditions, simply by an observation of the tension developed by it in a series of isometric twitches: which may render the result useful in investigating the energy exchanges of isolated muscles kept under various chemical or physical or nutritive conditions. I have used it to obtain an estimate of the total energy available to excitation, in a sartorius muscle kept in air, in oxygen, or in oxygenated Ringer's solution.

Pairs of sartorii have been kept in a moist chamber and stimulated either (*a*) every 2 secs. or (*b*) every 10 secs., by platinum electrodes, until they were completely inexcitable. The tension developed in each twitch was recorded on a slowly moving drum, and the total tension developed, multiplied by the length of the muscle and the appropriate constant, gave in calories the total heat liberated by the muscles. Hitherto only a few experiments have been performed, but the following are typical.

A. Sartorii in O_2. Twitch every 10 secs.

Exp. 1. Total tension developed in about 2000 twitches, 47,500 grms. wt. Length of muscles, 3·5 cm. Weight, ·286 grm. Calculated heat-production

$$=3{\cdot}5\times 47{,}500\times 10\times 10^{-6}/{\cdot}286 \text{ calories per grm.,}$$
$$=5{\cdot}8 \text{ calories.}$$

Exps. 2, 3, 4, 5, 6. 17·2 cals., 7·9 cals., 6·1 cals., 5·6 cals. and 47·0 cals. per grm.

B. Sartorii in air. Twitch every 2 secs. Recovery heat-production must have been negligible, so constant assumed was 5 microcalories and not 10.

Exps. 1 and 2. 1·38 cals. and 1·56 cals. per grm.

C. Sartorii in oxygenated Ringer's solution.

Exps. 1, 2 and 3. 37 cals., 33 cals. and 30 cals. per grm.

According to Peters the heat set free in stimulation to complete fatigue is about 0·9 cal. per grm., in the almost complete absence of O_2. The experiments (B) above were not in the complete absence of oxygen, and were on much purer muscle substance than were Peters' experiments. Considering this the agreement with Peters' experiments is rather good. We see however that in sufficient oxygen the value of the heat-production up to complete inexcitability may be enormously increased. The maximum value obtained in O_2—the muscle went on twitching five times a minute for nearly 48 hours—was 47 calories, which is nearly thirty times as large as the quantity of heat yielded in the production of the lactic acid maximum of rigor, and corresponds to the oxidation of approximately 1·2 % of carbohydrate. Muscle is stated to contain from ·4 to 1 % of carbohydrate, and the higher figure might be sufficient to account for all the heat given out. These experiments are apparently best performed in oxygenated Ringer's solution: for under such conditions one practically always obtains a high value, over 30 cals., corresponding to the oxidation of say 0·8 % of carbohydrate. In O_2 the muscles usually die rather quickly.

The high values obtained show that the heat-production is not due to the oxidation of lactic acid: there is far too little lactic acid present to account for all the heat.

The method may conceivably prove valuable for various purposes: it will be well to check it occasionally by direct myothermic experiments, or by estimations (by Fletcher's method) of the CO_2 set free.

A self-recording calorimeter for large animals. By A. V. Hill and A. M. Hill.

In a recent number of the *Journal of Physiology* (XLVI. p. 81. 1913) we published an account of a self-recording calorimeter for small animals, the principle of which was the use of a horizontal cylindrical Dewar flask cooled internally by a rapid flow of water, whose rise of temperature on passing through the calorimeter was recorded differentially and electrically by means of a thermopile and a self-recording d'Arsonval galvanometer: the heat-production was calculated as the product of the rate of water flow, and the *area* of the curve recording the rise of temperature. It was ascertained by preliminary experiments made by one of us (A. M. H.) that the principle could be applied completely to the construction of larger calorimeters, involving not a Dewar flask but simply a well-insulated iron-cylinder, cooled by a rapidly circulating stream of water. By the kindness and with the encouragement of Dr F. G. Hopkins and of Prof. T. B. Wood of the Cambridge School of Agriculture, who have provided us with a suitable underground room and apparatus, and with the aid of a grant made by the Board of Agriculture from funds placed at their disposal by the Development Commissioners, for research in Animal Nutrition, we have been enabled to construct a self-recording calorimeter of a size suitable for a small sheep or a large dog. It consists of two horizontal coaxial iron cylinders, the inner 4 feet long and 2 feet in diameter, the outer 4 feet 6 inches long and 3 feet in diameter. The space between them is filled with sawdust and capok wool, acting as heat-insulators: and "compo." tubing soldered spirally round them allows water to run, first around the outer and then around the inner cylinder, at about 700 c.c. per minute. It is usually not difficult to keep the water flow sufficiently constant. The rise of temperature of the water on passing around the inner cylinder is measured by a thermopile consisting of 16 couples of constantan and copper, which is insulated by silk-ribbon and pitch, and enters the ingoing and outgoing water through two T-pieces. The instrument requires no attention beyond measuring the water flow and the area of the temperature curve. A rise of 1° C. gives some 15 cm. deflection on the galvanometer drum, and this sensitivity is usually far greater than is necessary and may therefore be reduced by the introduction of an appropriate resistance. The heat-production is measured by the product,

K {Area of temp. curve in sq. cm.} {Water flow in c.c. per min.}

where K is some constant. The area of the curve is measured by a planimeter. The liberation electrically of a known amount of heat inside the calorimeter has led to the following calibration numbers for K, given in the order in which they were made [each represents an experiment lasting 24 hours]:—

4·55, 4·46, 4·60, 4·44, 4·68, 4·46, 4·52, 4·55, 4·56, 4·54,

with a mean value of 4·54, and an average divergence from the mean of 0·05, *i.e.* of about 1 %. These calibrations have seemed so satisfactory that we are now in process of erecting a larger calorimeter for men, cattle, or horses, on almost identically the same plan: this we hope will be completed in one or two months.

It is generally supposed that the construction of a large calorimeter (*e.g.* of an Atwater instrument) is exceedingly expensive, and its employment elaborate and difficult. It is noteworthy therefore that —apart from the self-recording galvanometer[1], which we had already— the preparation of the room has cost about £25, the calorimeter already constructed has cost about £20, and the large calorimeter now under construction (9 ft. 6 in. long and 5 ft. 6 in. high) is estimated to cost approximately £40. The accuracy attainable by the large calorimeter is at present unknown, but should be at least as great as that of the smaller instrument: and the making of the actual calorimetrical observations takes exceedingly little time, involving only the measurement of the water flow and the temperature curve, as described above. Attention of course will have to be paid, as in all actual experiments on live animals, to the amount of evaporation and to the heat carried away by the air current.

---

[1] But for the trouble of recording the deflection continually any fairly sensitive low-resistance galvanometer would serve admirably.

A simple and convenient form of bicycle ergometer. By C. J. MARTIN, *Lister Institute.*

The ergometer is of the brake-type. The work done in foot pounds is calculated from the frictional pull, the revolutions and the circumference of the wheel. Although this type was formerly used by physiologists, it has been abandoned of late years in favour of more complicated devices, such as driving a dynamo from the bicycle and measuring the voltage and current developed (Atwater), or substituting a copper disc for the hind wheel, which is run between the poles of an electro magnet, thus constituting an electric brake (Krogh, Benedict and Carpenter). The present form was devised for experiments in hot or moist atmospheres extending over considerable periods and the principal ends aimed at were moderate accuracy and independence of any assistance, that is, the experimenter working the bicycle to be able to make all the necessary observations.

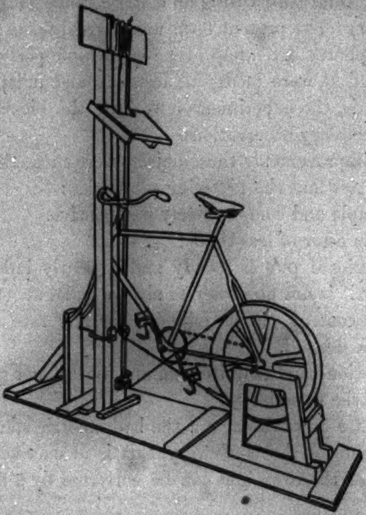
By gradual improvements these objects were more than attained and the instrument now has the following properties:

1. It is simple and comparatively inexpensive.
2. It has an error of less than 1 %.
3. The frictional pull is steady and is nearly independent of the velocity, so that it is not necessary to always pedal at a constant rate[1].
4. The instrument can be adjusted for any rate of work at any pedal-revolution desired.
5. The friction-band is quite thin and so does not get hot and vary the pull during the experiment.
6. Should for any reason, *e.g.* the hydrometric state of the atmosphere, the tension of the cords vary, this is at once seen, the balances being in front of the eyes, and can be corrected by a fine screw movement.

The general design of the apparatus will be seen from the accompanying woodcut. It consists of a bicycle frame supported by a wooden stand, from the front of which two uprights ascend and carry a small desk, and a cross piece which provides for the attachment directly in front of the worker of the balances and other pieces of apparatus which may be necessary. A cast-iron wheel, 5½ feet in circumference, weighing 22 kilos. and mounted on ball bearings, is substituted for the

[1] A frictional pull of 5 lbs. increased by half an ounce when the velocity of pedalling varied from 20 to 200 per minute *i.e.* 0·6 %.

back wheel. This wheel is one inch wide at the periphery, has slightly bevelled edges and is absolutely true. To the circumference of the wheel a thin band of linen or stout calico (such as a fracture bandage) with hemmed edges is applied and the two ends of the band are attached by whipcord to spring balances, having a range of 6 lbs. and graduated to one ounce, attached to a board on the top of the uprights. Hemming the edges of the bandage makes them slightly shorter than the centre and ensures the belt keeping in position on the fly-wheel. The cords to the balances pass round ball-bearing aluminium pulleys[1] in order to give them the right direction. These pulleys are mounted on blocks of wood which are attached to the lower part of the uprights by clamps.



In using the instrument the tension of the cord is roughly adjusted by moving the pulleys up or down, so as to provide the friction required; the final adjustment is made by turning the screw of a slide rest to which one of the balances is attached. The revolutions of the wheel are recorded by a cyclometer.

---

[1] Such as are used for making simple Atwood machines for schools, cost, 3s. 6d.

The electrical axis of the heart in systole and in diastole.

By A. D. WALLER.

In previous communications it has been demonstrated that the value of the inferior angle formed with the vertical by the electrical axis of the heart can be estimated from the formula $\tan \alpha = 2\frac{R-L}{R+L}$ where R and L are the magnitudes of the first ventricular wave V_I of the right and left hand inferior leads. This first ventricular wave V_I, or systolic spike, lasting about 0·04 sec., precedes the mechanical systole by a brief interval of about the same duration, so that strictly speaking it is presystolic in relation to the mechanical event, and is manifested during the relaxed state of the ventricles. The position of the electrical axis calculated from the R and L values of V_I is therefore taken during the diastolic state of the ventricles.

Similarly the second ventricular wave V_{II}, of more prolonged and less definite duration, occurs during ventricular systole, and comes to an end just before the occurrence of the second sound. Its relative amplitudes on the right and left sides may be taken into calculation for the estimation of the axial inclination during the systolic state of the ventricles. Thus *e.g.* in a typical case (M. L. B.) we have the following values:

	Right	Left
V_I	9	6
V_{II}	6	2

$$\text{from which for } V_I \quad \tan\alpha = 2\frac{9-6}{9+6} = \frac{6}{15} = 0{\cdot}4, \quad \therefore \alpha = 22^\circ,$$

$$\text{,, \quad ,, \quad ,, } V_{II} \quad \tan\alpha = 2\frac{6-2}{6+2} = \frac{8}{8} = 1, \quad \therefore \alpha = 45^\circ.$$

In this case where the heart approximates to the vertical type, it is evident that the electrical axis for V_{II} during ventricular contraction forms a wider angle with the vertical than the similar axis for V_I during ventricular relaxation.

With hearts of the horizontal type, the difference between the electrical axes during systole and diastole comes out differently. Thus *e.g.* in the next case (A. D. W.) with

		Right	Left
V_I	=	9	−15
V_{II}	=	6	−3,

we have:

$$\text{for } V_I \quad \tan\alpha = 2\frac{9+15}{9-15} = \frac{48}{-6} = -8, \quad \therefore \alpha = 180-83 = 97^\circ$$

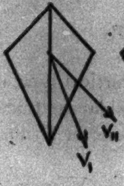
$$\text{for } V_{II} \quad \tan\alpha = 2\frac{6+3}{6-3} = \frac{18}{3} = 6, \quad \therefore \alpha = 80^\circ.$$

In this case of approximately horizontal heart with both ventricular waves negative in the left hand lead, it is clear that the electrical axis for V_{II} during ventricular contraction forms a smaller angle with the vertical than does that of V_I during ventricular relaxation.

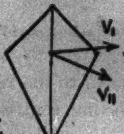
Cases 3 and 4 are those of a brother and sister, both normal, of whom I took records a few weeks ago at the Institut Marey, and who happen to afford typical instances of vertical and horizontal types respectively. The axis of V_{II} is further from the vertical than that of V_I in the former, further from the horizontal in the latter, *i.e.* in both cases the electrical axis during systole approaches the midway position between 0° and 90°, viz. 45°.

		V_I (ventricles relaxed)			V_{II} (ventricles contracted)		
		Right	Left	α	Right	Left	α
1.	M. L. B.	9	5	22°	6	2	45°
2.	A. D. W. (in Exp.)	9	−15	97°	6	−3	80°
3.	M^{lle} X.	4	−5	93°	3·5	−1	74°
4.	M. X.	12·5	12·5	0°	4	2·5	25°
5.	T. H. K.	12	8	22°	4	2·5	25°
6.	Prof. C. R. (in Insp.)	13	−5	77°	6	4	22°
7.	B. O. B.	18	6	45°	6	2	45°
8.	J. C. W.	14	7	34°	3	1	45°
9.	Dr M.	18	16	7°	6	3	34°

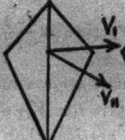
1. M. L. B.



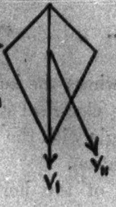
2. A. D. W.



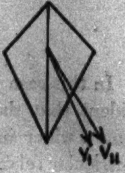
3. M^{lle} X.



4. M. X.



5. T. H. K.



PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

March 14, 1914.

Some effects of barium chloride. By W. BURRIDGE. (*Preliminary communication.*)

The tonic contraction produced by barium chloride in the frog's ventricle might be reproduced many times (30) in the same heart. The contraction is termed a tonic contraction because it subsided in presence of the salts evoking it, and showed a greater dependence on the condition of the exciting mechanism than it did on the condition of the contractile mechanism. The contraction produced by a given concentration of barium chloride in the same heart might vary greatly where the contraction produced by a sufficiently concentrated potassium chloride solution was constant.

The barium contraction was directly antagonised by calcium salts, but any antagonism by potassium salts was doubtful.

Barium salts might apparently replace calcium salts as regards the induction of tonus but not as regards the formation of semi-permeable membranes.

After treatment of the heart by barium salts the state of aggregation of certain of the heart colloids was found to be altered. This alteration was the same as that induced by calcium, and was one unfavourable to the further functional activity of calcium in regard to support of the spontaneous contractions, etc. Appropriate treatment with sodium or potassium salts restored the functional activity of calcium.

The preparation of pancreatic extracts. By J. MELLANBY and V. J. WOOLLEY.

It has been found that in order to prepare from the pancreas solutions containing the various constituents of pancreatic juice different extractives must be used.

Trypsinogen may be prepared by extracting finely divided fresh pancreatic tissue with twice its weight of ·5 °/₀ HCl at room temperature for twelve hours. This extract when neutralised by $NaCO_3$ and filtered from the precipitated acid albumen gives a water clear solution which contains an abundance of trypsinogen. Such an extract preserved under toluol remains inactive for weeks at room temperature since the acid destroys the greater part of the enterokinase contained in the gland.

Trypsin may be obtained by adding 1 °/₀ enterokinase (a filtered water extract of the mucous membrane of the upper part of the small intestine) to the above solution thus:

		Units of trypsin after			
Trypsinogen	E	5′	10′	15′	20′
1 c.c.	·1	0	180	1000	2000

The course of activation is the same as that observed in fresh pancreatic juice.

Trypsinogen may also be prepared by extracting pancreatic tissue with twice its weight of glycerine. This method is not so satisfactory since glycerine inhibits activation by enterokinase.

			Units of trypsin after				
Glyc. extr.	E	H_2O	15′	25′	50′	90′	180′
·5 c.c.	·1 c.c.	—	—	120	150	220	1000
·5	·1	4·4	2000	—	—	—	—

Dilution with water removes this inhibitory influence. With this extract also the course of activation is the same as that observed in fresh pancreatic juice.

Steapsin. In the preparation of this ferment it is essential that no trypsin should be developed in the solution since, as we have shown, trypsin immediately destroys lipase. Extractives which inhibit the formation of trypsin are glycerine and sodium carbonate. From our experiments we find that glycerine extracts are unsatisfactory and that the most certain way of preparing a steapsin solution is by extracting fresh pancreas with twice its weight of ·5 °/$_0$ Na_2CO_3 for twelve hours. The following figures indicate the activity of such an extract:

Olive oil emulsion (50 °/$_0$)	H_2O	Steapsin extract	NaOH $\left(\frac{N}{10}\right)$ required to neutralise acid after 1 hour
2 c.c.	·5	·5	2·4 c.c.

Amylopsin. The most efficient extractive for this ferment is glycerine. The activity of a preparation made by extracting fresh pancreas with twice its weight of glycerine for twenty hours at 37° C. was such that ·1 c.c. rendered 3 c.c. of 1 °/$_0$ starch achromic in one minute. The activity of the ferment is greatly increased by the presence of small quantities of HCl or NaCl in the digestion mixture.

The influence of acetoacetic acid on the estimation of creatinine. By E. P. Cathcart and J. B. Orr.

Last year Greenwald(1) pointed out that the presence of acetoacetic acid in the urine interfered very materially with the estimation of creatinine. Recently Graham and Poulton(2) confirmed this observation and asserted that, when the carbohydrate metabolism is upset, the acidosis which resulted wholly accounted for the difference in the colorimeter readings between the preformed and the total creatinine. We have fully confirmed the original observation of Greenwald but we cannot substantiate the claim of Graham and Poulton.

The following figures for starvation show that the acidosis does not wholly account for the "creatine" output.

Day of starvation	Preformed creatinine plus aceto-acetic acid	Preformed creatinine minus aceto-acetic acid	Total creatinine	Creatine
I	1·47 grm.	1·53 grm.	1·56 grm.	·03 grm.
II	1·41	1·48	1·59	·11
III	1·39	1·47	1·59	·12

Further evidence for the association in some fashion of creatine with carbohydrate metabolism is offered in phloridzin glycosuria. The following results were obtained after the injection of 1 gram of phloridzin into a 12 kilo dog.

Total creatinine	Preformed creatinine minus aceto-acetic acid	Creatine	Total acetone and aceto-acetic acid	
·352 grm.	·352 grm.	·0	5·3 mgrm.	Average output for 8 pre-days.
·405	·365	·040 grm.	10·0	Injection of 1 grm. phloridzin.
·375	·358	·017	5·1	
·360	·345	·015	—	

The results of Wolf and Osterberg(3) fully confirm this observation.

We have also carried out a series of experiments on the influence of carbohydrate on the output of creatine after the injection of sodium selenite. The following Table is taken from one of our experiments.

It may be remarked in conclusion that the method suggested by Graham and Poulton for the removal of the acetoacetic acid before carrying out the creatinine estimation is not so simple and easy as they infer. Unless extreme care be taken too low readings are obtained, the result apparently of some destruction of the creatinine.

Total creatinine	Preformed creatinine minus aceto-acetic acid	Creatine	Total acetone and aceto-acetic acid	
·433 grm.	·377 grm.	·057 grm.	4·6 mgrm.	Average output for 3 pre-days.
·640	·413	·227	5·4	Injection of selenite.
·644	·404	·240	8·1	
·458	·385	·073	5·6	

(1) **Greenwald.** Journ. of Biol. Chemistry, XIV. p. 87. 1913.
(2) **Graham and Poulton.** Proc. Roy. Soc. LXXXVII. p. 205. 1914.
(3) **Wolf and Osterberg.** Amer. Journ. of Physiol. XXVIII. p. 71. 1911.

The right lateral auriculo-ventricular junction of the heart. By A. F. STANLEY KENT.

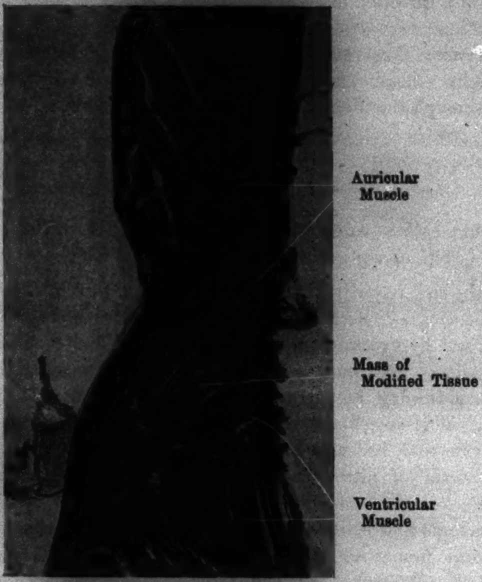
(*From the Physiological Laboratory, University of Bristol.*)

In a former communication to the Society (*Proc. Phys. Soc.* November 15th, 1913, and *Journal of Physiology*, XLVII. Nos. 4 and 5) it was pointed out that specialized muscular tissue may be found in situations other than the sino-auricular node and the auriculo-ventricular node, and that one such situation is near the auriculo-ventricular groove on the right lateral aspect of the heart. The close association between the specialized tissue and the ventricular muscle was noted.

A further note, accompanied by a photograph, was published in the *Quarterly Journal of Experimental Physiology*, VI. No. 2.

The microscopical preparations, photographs, and drawings which accompany this communication show some of the relations of the specialized tissue in this situation.

The specialized tissue is seen to occupy the centre of a mass 0·9 mm. in length by 0·25 mm. in breadth. The outer part of the mass is composed of muscular fibres which resemble the tissue of the auricle and which become continuous with it, the fibres passing generally in a direction at right angles to the A.-V. junction. The tissue in the centre of the mass is of the modified type. The mass is in close association with the auricular musculature. It is separated from the ventricular fibres for some part of its length by connective tissue which consists of comparatively coarse bundles of fibres with numerous cells lying between them. This connective tissue may be 0·05 to 0·1 mm. thick. On its ventricular aspect it is penetrated by the finely tapering bundles of ventricular muscle.



Right Lateral A.-V. Junction in Man. × 30.

The connective tissue septum may be reduced still further in thickness, and may become discontinuous. In some of the preparations shown it will be noticed that it is finally penetrated by groups of fibres directly continuous with the ventricular muscle. These muscular fibres having penetrated the connective tissue septum become connected with the substance of the above-mentioned mass of specialized tissue.

The ventricular fibres which take part in the connection are derived from two sources, (*a*) fibres lying near the inner aspect of the ventricular wall, and running up from the direction of the apex, and (*b*) fibres derived from a mass of ventricular substance lying more deeply and running in a direction more nearly parallel with the plane of the A.-V. junction.

Conclusion. The ventricular muscle of the right lateral wall of the heart (right ventricle) is connected with the auricular muscle (right auricle) through the intermediation of the modified tissue described.

This research has been assisted by a grant from the Research Fund of the University Colston Society.

Choline-esters and muscarine. By H. H. DALE and A. J. EWINS. (*Preliminary communication.*)

We showed recently that the principle in certain ergot extracts having an action conforming generally to the "muscarine" type, but characterised by extraordinary rapidity, potency and evanescence, was acetyl-choline. For comparison a quantity of the "synthetic muscarine" of Schmiedeberg and Harnack ("pseudomuscarine") had been prepared. This substance, which is obtained by heating choline with strong nitric acid, has now been shown by one of us (A. J. E.) to be a choline-ester of nitrous acid, and not an aldehyde as previously supposed.

The nitrous acid ester, completely identified chemically, has all the physiological properties ascribed by previous workers to "pseudomuscarine." It causes all the typical muscarine effects, except constriction of the mammalian pupil; on the other hand, it has potent actions of the nicotine-curare type which natural muscarine does not possess, and which are not annulled by atropine. The discrepancy between the action of the synthetic and natural "muscarines" is explained by the correct identification of the synthetic product. At the same time the constitution of the natural muscarine becomes once more an open question, since the evidence for the aldehyde formula has practically vanished. Working on the idea that it, too, is probably a choline-ester, or mixture of esters, we have found that the nitric-acid ester has all the actions of natural muscarine on the mammal, including the intense myotic action. Quantitatively its action on the frog's heart resembles that of the nitrous-acid ester, being weaker than that of muscarine; the difference, however, is readily adjusted by adding a very small proportion of acetyl-choline. Such a mixture is indistinguishable in action from an Amanita extract, except in possessing a nicotine-curare action on the atropinised frog, which the natural muscarine has not. It still seems possible, therefore, that the series of choline-esters will be found to contain "natural" muscarine also.

It should be noted that the choline esters hitherto examined, while showing the peripheral "muscarine" action in small doses, show in larger doses, like many quaternary ammonium bases, a nicotine-like action, unaffected by atropine, but abolished by nicotine. Choline itself shows both types of action in much weaker degree, and this duplicity of action has been the source of not a little controversy, as to whether pure choline was "depressor" or "pressor" in its action on the mammalian blood-pressure.

The action of hydrazine hydrate on methæmoglobin. By GEORGE A. BUCKMASTER.

(*From the Institute of Physiology, University College, London.*)

The hydrazine method for determining the oxygen-content of blood gives values which are identical with the ferricyanide method of Haldane and Barcroft[1].

The hydrazine method can be employed to ascertain the quantity of oxygen which is liberated from methæmoglobin when this is converted by removal of its oxygen into reduced hæmoglobin.

It has been shown by E. Letsche[2] that hydroxylamine added in definite quantity to oxyhæmoglobin completely converts this to methæmoglobin (neutral solution), the quotient of the two extinction-coefficients being 1·185. Such a quotient is proof that practically all the oxyhæmoglobin is converted into methæmoglobin. The possible error is less than 2·5 per cent.

Neutral solutions of methæmoglobin prepared in this way when reduced with hydrazine hydrate and the nitrogen evolved measured never yield an amount more than half that given by the same quantity of oxyhæmoglobin. The reduced hæmoglobin which is produced can now be re-oxygenated at 0° C. and the oxygen-content again determined.

---

[1] Buckmaster. *Proc. of Physiol. Soc.* June 28, 1913.

[2] *Zeits. f. physiol. Chem.* LXXX. p. 419. 1912.

Example.

Fresh defibrinated dog's blood is saturated with oxygen.

	c.c. per 100 c.c. blood
Total oxygen-content	19·1
„ with hydrazine hydrate	19·0

Exactly converted into methæmoglobin by hydroxylamine (50 c.c. of blood with 14·1 % Hb requires 9·3 N/10 hydroxylamine hydrochloride neutralised with N/10 NaHO).

	c.c. per 100 c.c. blood
Total oxygen-content of methæmoglobin by hydrazine hydrate	8·4
„ after re-oxygenation at 0° C. by hydrazine hydrate ...	14·8

A number of similar experiments show that in no case can a volume of oxygen be obtained from methæmoglobin which is more than half that yielded by oxyhæmoglobin.

Since the gas yielded both when oxyhæmoglobin and methæmoglobin are reduced by hydrazine hydrate is nitrogen equivalent in volume to the oxygen-content, it would appear that the view originally held by Hoppe-Seyler[1] and more recently by Küster[2] and Letsche that methæmoglobin contains a less quantity of dissociable oxygen than oxyhæmoglobin is correct.

---

[1] *Zeits. f. physiol. Chem.* I. p. 396. 1877–8 and II. p. 152. 1878–9.

[2] *Ibid.* LXVI. p. 247. 1910.

Demonstration of the negative after-images of spectral and compound colours of known composition. By F. W. EDRIDGE-GREEN and A. W. PORTER.

The colours are produced with the colour box of A. W. Porter and projected on a screen in a dark room. The spectrum is divided into two portions so that two colours are seen on the screen, one a simple spectral colour and the other formed by the remaining rays of the spectrum. Where the two overlap white is produced. These two coloured images are viewed intently for 10 seconds and then the eyes are directed to another portion of the screen. It will be noted that the coloured after-images are not the true complementaries of the original colours viewed. We intend to measure these colours by means of a comparison spectrum.

Blood-pressure estimation by the auditory method. By J. A. MacWilliam, G. Spencer Melvin, and J. R. Murray.

At a comparatively early stage in our investigation of methods of blood-pressure estimation in man, we had recourse to animal experiment, though we have not hitherto published any statement of such experiments having reference to the verification of diastolic pressure estimation by the auditory method.

In the case of small animals, the testing of the auditory method is not satisfactory, but in animals of sufficient size the procedure offers no special difficulty apart from those connected with respiratory disturbance associated with the anæsthetic. We have found the sheep a very suitable subject. The results are very definite and convincing.

The animal is anæsthetised with ether and chloroform, after a preliminary injection of morphia and chloral. A large-sized reservoir cannula is placed in the central end of the left carotid artery at the root of the neck, and is connected, by non-distensible tubing, with a set of manometers—valved ones for maximum and minimum pressures and a "compensated" one for mean pressure—so that the intra-arterial pressure can be brought to bear on any of the manometers as desired.

An ordinary armlet 12 cm. broad is applied round the neck, it being noted that the circumference of the latter is not too great for such a breadth of armlet. The armlet pressure being raised and lowered in the usual way, the auditory phenomena are examined by means of an Oliver tambour (phonendoscope) applied over the right carotid artery on the distal side of the armlet, the artery being left covered by the tissues with the exception of the skin.

In order to prevent disturbance from venous congestion of the head due to obstruction caused by the armlet pressure, the internal jugular vein is protected by a split lead tube (15 cm. long) which prevents its being compressed by the armlet pressure. A tracheal cannula was sometimes used.

One observer made the auditory observations and read off the armlet pressures from a mercury manometer, while another determined the intra-arterial pressures as shown by the valved manometers. The observers frequently changed places and repeated the estimations. The operative part of the experiments was performed by two of us (J. A. MacW. and G. S. M.).

The auditory phenomena were very marked and characteristic, the phases corresponding with those found in man and in a circulatory schema. The systolic auditory index was found to be correct. The auditory diastolic index constituted by a sudden dulling and weakening of the sound at a certain point in the lowering of the armlet pressure proved a very accurate guide to the intra-arterial diastolic pressure as shown by the minimum manometer[1]. Extinction of sound was much later.

Considerable variations in pressure and pulse rate were induced in the course of the observations with no impairment of the accuracy of the results. The pressure was raised by mechanical compression of the abdomen, lowered by an increased dose of chloroform etc. Pulse rates varying from about 60 to 110 were present at the different phases.

We also tried the oscillatory method by connecting the armlet with the Erlanger sphygmomanometer and with the Pachon sphygmo-oscillometer, but found the indications more difficult to read than the auditory ones.

At the beginning of the observations before the armlet was inflated the pressures, as shown by the manometers, were Systolic 110, Mean 80, Diastolic 52; the mean pressure being only slightly different from the arithmetical mean.

Some experimental data are subjoined:

	Intra-art. press. by valved manometers			
Observation	Systolic	Diastolic	Auditory diastolic reading	Remarks
1	96	45	45	Much chloroform given.
2	96	51	50	—
3	98	54	50	—
4	96	56	53	—
5	96	60	60	—
6	102	84	80	Compression of abdomen. Pulse rate 110.
7	96	60	60	—
8	112	70	70	Compression of abdomen.
9	112	70	70	—
10	85	41	40	Dose of chloroform increased.
11	85	48	47	—
12	94	46	45	—
13	94	74	74	—
14	94	65	68	—
15	80	56	54	—

---

[1] We arrived at this conclusion on other grounds also, from experiments with a circulatory schema as described in *Heart*, 1914, v. 193, and from observations made on man in different circumstances—see a paper in the *Quart. Journ. of Exp. Physiol.* 1914, viii. and one in the *Brit. Med. Journ.* March, 1914.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

May 16, 1914.

The phenomenon of augmentation of excitability in the motor cortex. By T. GRAHAM BROWN (*Manchester*).

Stimulation of a motor point in the cortical area demonstrates, as is well known, the phenomenon of facilitation. With repetition of stimulation the movements become more easily evoked and of greater strength. Not only is this the case, but the area from which the movements may be evoked appears to increase so that they are obtainable from a point which previously was irresponsive. This phenomenon became of importance in connexion with a seeming activation of the post-central cortex which I have observed. The question is: if a point during stimulation increases in excitability, may the apparent excitability, then, of another point, which previously was irresponsive, be explained simply as due to spread of current in stimulation—the component of the overflow which then reaches the first point being now effective? The alternative is that the whole surrounding cortex takes part in this increased receptibility, and that the activation of a previously inactive point is a physiological phenomenon and conditioned by its direct stimulation.

In investigating this the method used is to take a strength of stimulus which is only just liminal. Applied thrice for periods of 1″ with 1″ intervals, it is found that an ordinary 'flexion-point' is usually rendered very excitable. The same stimulus is then (within one or two seconds) applied to a point on the motor cortex which does not yield flexion at that strength of stimulus. But it is found that often after this activation of the flexion-point the hitherto irresponsive point is excitable and reacts with the same type of reaction with which the flexion-point responded.

I have then used a method for the rapid destruction of parts of the motor cortex. Liquid air is directly applied and small areas are frozen within a few seconds. They are frozen until quite hard, and are then as rapidly thawed again. If care is taken not to injure the blood vessels the appearance of the cortex after freezing is almost normal, and the whole procedure may be performed very rapidly.

It appears, in some cases at least, that after this rapid freezing the activation of the hitherto irresponsive point immediately after the facilitation of the flexion-point no longer takes place. This seems to be the case where the strength of stimulus used is only just liminal. The rapid freezing and thawing can hardly be supposed to alter the electrical conditions of conductivity of the cortex. But, in any case, that it is not due to such a change is shewn by the fact that other (unfrozen) hitherto irrresponsive points which lie at the same, or even at a greater, distance from the flexion-point are found to be capable of activation after the one point is frozen.

This phenomenon of the spread of the area of facilitation in the cortex cannot therefore be explained on the assumption that a single point is facilitated and the other phenomena are due to the consequent rise in the value of the over-flow of the stimulating current which is applied to other points. It must rather seem that a wide area of the cortex takes part in the facilitation of any one act, and that then hitherto irresponsive points within it, or points which previously gave another reaction, become responsive and in the sense of the movement the elicitation of which was primarily facilitated.

Motor activation of the post-central gyrus. By T. GRAHAM BROWN (*Manchester*).

Although, since the work of Grünbaum and Sherrington, it has been generally recognised that the motor cortex in man, in the apes, and in monkeys, does not normally extend back into the post-central gyrus, it is yet possible that under certain circumstances motor reactions may be obtained from that area of the brain.

In investigating this point it was found that after the activation of a regular flexion-point in the motor area of monkeys, for a short time stimulation of the post-central gyrus apparently evoked similar movements. It was found that this state of activation of the post-central gyrus might occur after separating it from the motor cortex by a longitudinal ditch parallel to the fissure of Rolando. In other cases the activation was apparently abolished by the same lesion. It was naturally supposed that the phenomenon might be due to spread of current which thus might stimulate the flexion-point in the motor cortex when the excitability of that point was raised.

But lately in using a method of rapid freezing of the cortex with liquid air some evidence has been obtained which appears to shew that this activation of the post-central cortex is physiological. A point in the post-central cortex which before demonstrated the phenomenon of activation may no longer do so after having been frozen. This is the case even where other and un-frozen post-central points still exhibit the phenomenon of activation after facilitation of the flexion-point in the motor cortex; and even where hitherto irresponsive points on the motor cortex itself shew activation, although they are at a considerably greater distance from the primary flexion-point than is the frozen point in the post-central cortex.

The phenomenon is therefore not due to spread, in all cases at least. Of course, if very strong stimuli were used it in part would be due to spread of current. The phenomenon of the activation of the post-central cortex is, in part at any rate, physiological. A question arises—how is this activation mediated? There are at least two possibilities. Of these the first is that the post-central centres are in a path of which the motor centres are also a part, but that they are more central than these centres, and act straight upon them and secondarily upon the lower centres through the pyramidal tracts. The other possibility is that there is an efferent tract from the post-central gyrus itself. I have no evidence to discriminate between these two possibilities, both of which, of course, may be actualities. The fact that the activation has been observed to outlast the lesion parallel to the central fissure speaks for an efferent tract from the post-central gyrus, and would seem to put the facilitation phenomenon in part at least in the sub-cortical centres. But the phenomena here might have been due to spread of current. The other observation that the activation does not in some cases outlast this lesion speaks for the effect of activation being carried out by the post-central gyrus, in part at any rate, through the motor cortex. But it might be that the lesion divided possible efferent fibres of the post-central gyrus in this experiment.

A respiratory tract in the mid-brain. By T. GRAHAM BROWN (*Manchester*).

The observations of Marckwald and others have suggested the presence, in the mid-brain, of a centre which in some manner regulates respiration, and probably lies in the formatio reticularis at the level of the posterior colliculi. That respiration may be affected from the higher centres—for instance, as a voluntary act—has, of course, long been recognised.

In exploring the cut surface of the mid-brain in monkeys I have not seldom obtained an alteration of the respiratory movements, and in a recent instance I have been able to localise the area from which this reaction may be obtained.

In this individual—*Cercopithecus callothrix*—it was found that when a point on a cross-section of the mid-brain through the anterior part of the anterior colliculi was stimulated with rapid faradic shocks in the unipolar method there occurred rapid 'gasping' or 'panting' movements. The abdominal muscles contracted and relaxed rhythmically and very strongly about once every second. The reaction at once ceased on withdrawal of the stimulus—there being no evident 'after-discharge.'

A point of interest is the extreme nicety of localisation which was possible in this case. The point itself lay about 3 mm. from the aqueduct directly to the side of it, but a little further back on the surface of the cross-section. With the strength of stimulus used—which gave very vigorous movements—stimulation applied all round this point, at a radial distance of 1·5 mm. only, was quite ineffective.

This extremely exact localisation of the reaction suggests a specific respiratory tract which may pass down and affect the lower respiratory centres. The point on the surface of the mid-brain which gave the reaction appeared (to examination with the hand lens) to coincide with a point on the efferent tract (tecto-bulbar ?) of the anterior corpora quadrigemina, which were only just involved in the cross-section. But no exact description of the histological relations of the point can as yet be made.

Note on the functions of the post-central gyrus in the anthropoid ape. By T. Graham Brown (*Manchester*).

I destroyed the post-central gyrus opposite arm and leg areas on the left cortex cerebri in a young chimpanzee on March 23, 1914. The area was 'ditched' and undercut between the ditches. It was also undercut for about 2 mm. into the motor area across the central fissure.

Seventy minutes after stopping narcosis in the operation the ape was found swinging himself hand over hand from the bars on the roof of his cage, and without using his feet. There appeared to be weakness or incoördination of the movements at right wrist and finger-joints—nowhere else. There was no detectable difference of sensibility in the two arms to gentle pressure, tickling, etc. The right arm was moved in voluntary trained movements and was with apparent accuracy directed to seize objects while the animal looked neither at the arm nor at the object.

On the third day after the operation the slight weakness of movement in right wrist and fingers had disappeared, and the movements of the animal could only be described as perfectly normal.

Mechanical efficiency of man. By J. S. Macdonald.

The heat-production of one of my subjects, E. J. Briscoe, cycling at revolution rates per min. 40–98, and performing external work upon a brake varying in different experiments from 0–73 kalories per hour is covered very closely by the following formula

$$\underset{(1)}{43V^{\frac{V}{251}}} + \underset{(2)}{\frac{56\cdot 3}{W^{\frac{1}{3}}}} \times \text{work in kals. per hour} = \text{kals. per hour.}$$

Of these two fractions, (1) and (2), the first is the heat-production associated with movement and is the same no matter what the value of the external work performed by the movement; the second is the 'coefficient of efficiency' multiplied by the external work done and fully represents the heat-production associated with the performance of external work.

It will be noticed that the 'coefficient of efficiency,' which is the reciprocal of the 'efficiency,' varies inversely with $W^{\frac{1}{3}}$; *it follows that the 'efficiency' varies directly with* $W^{\frac{1}{3}}$. This direct variation of the 'efficiency' with the subject's weight, but less rapidly than this weight and precisely with $W^{\frac{1}{3}}$, is common to all the subjects I have examined, and the influence of the same fact is evident in experimental evidence collected by others.

The first part of the expression, (1), namely the heat-production associated with movement,

$$43V^{\frac{V}{251}} \text{ kals. per hour,}$$

where V is the revolution rate per minute, is when changed as follows applicable to my other subjects when cycling at, in their cases, the only one rate which I have so far examined (60 per min.)

$$\boxed{\cdot 87WV^{\frac{VW^{\frac{1}{3}}}{1878}}.}$$

This expression for heat-production associated with movement, in my opinion, owes its nature to the pendular character of the limb movements and their possession of an optimum rate of movement associated with a minimum heat-production per movement at that particular rate.

Whilst formulating the results of my own experiments I have sought for analogies in the experimental observations of others and have found the following, in my opinion, interesting fact. Douglas 'walking on the grass at Oxford' (Douglas and Haldane, etc. *Phil. Trans.* B, CCIII. p. 245 etc.) presents results which may be expressed as follows:

$$134S^{\frac{S}{505}} \text{ kals. per hour,}$$

where "S" equal the number of strides per minute. When attention is paid to the fact that in cycling each leg performs a 'double oscillation' returning at the end of each revolution to its original starting point, whereas in walking it takes two strides to complete a similar cycle, each stride being a 'single oscillation'; then this formula for Douglas 'walking on the grass at Oxford' becomes

$$134V^{\frac{V}{253}} \text{ kals. per hour,}$$

where "V" represents the number of 'double oscillations' per minute. In this form it is obviously analogous to Briscoe's cycling formula, differing from it only in possessing a different limiting value, 134 in the one case and 43 in the other. These limiting values represent the 'dynamic postures' of walking and cycling respectively.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

June 6, 1914.

Improved myothermic apparatus. By A. V. HILL and VIKTOR WEIZSÄCKER. (*Demonstration.*)

All myothermic experiments made hitherto on isolated muscles have been carried out in air, oxygen or nitrogen. We have developed a method for working on muscles immersed in Ringer's solution, which is thoroughly simple, and offers various advantages. We use a thermopile of the form described by A. V. Hill (This *Journal*, XLVI. p. 42. 1913), a "long straight thermopile" with 35 pairs of junctions. The thermopile contains muscle holder and stimulating electrodes complete. The thermopile holding the muscle is fixed to a long glass rod, and held near the bottom of the inside of a cylindrical Dewar flask of 40 cm. depth and 8 cm. diameter. Enough Ringer's solution (500 c.c.) is introduced to cover the muscle and thermopile. A wooden stopper holds the glass rod and closes the Dewar flask. Through holes drilled in the stopper go (1) the insulated leading-off wires of the thermopile, (2) wires for electric excitations, (3) a thread to the end of the muscle for recording isometric contractions, (4) a stirrer and (5) a tube for changing the Ringer's solution. The chief advantages and peculiarities of this arrangement are as follows: (1) rapid attainment of temperature equilibrium: (2) easy maintenance of a constant temperature, whether at, above or below the room temperature: (3) possibility of a rapid alteration of physical or chemical conditions, by changing the Ringer's solution: (4) very much better conditions for the muscle, which lives far longer in the Ringer's solution than it ever will do in air.

The apparatus has been used to estimate heat-production both in single twitches and in tetani. The heat-capacity of water is some 3000 times greater than that of air: its conductivity somewhat greater. Both factors cause a rapid and great heat loss from the muscle to the surroundings, and it is found in practice that the galvanometer deflections are reduced 5 to 10 times by immersing the muscle in the Ringer. Nevertheless, the 100 mm. or so obtained for a single twitch is quite sufficiently large, and we have found that we can obtain a sensitivity amply sufficient for all purposes. In consequence of the greater heat-capacity of the convection currents set up in water, the galvanometer is not so steady as when the thermopile is in air. The investigations made therefore have confined themselves hitherto to the *initial* heat-production.

It should be distinctly understood that these experiments are not calorimetrical: the rise of temperature of the muscle immediately warms the thermopile in contact with it: the heat is not collected and measured as in a micro-calorimeter.

The effects of various physical and chemical factors on the initial heat-production of muscle. By VIKTOR WEIZSÄCKER.

By the method communicated by A. V. Hill and myself, myothermic experiments can be carried out with muscles immersed in various solutions. The chief results obtained so far by this method are as follows. In the first place it should be noticed that a rapid change of the solution around the muscle can be effected, and it is usually possible within a few minutes of changing the solution to make observations of the heat-production. Thus successive observations on the muscle, under various conditions, are rendered possible before the general condition of the muscle has had time to change appreciably. It has been found that the "initial heat-production" (as represented by the maximum deflection of the galvanometer) is exactly the same in Ringer's solution (*a*) saturated with, or (*b*) free from oxygen. Also, when oxidations are prevented by the presence of KCN the initial heat-production is unaltered. It is therefore very likely that the initial heat-production is entirely due to a non-oxidative process. Thus one is enabled to perform a separate study of each of the two main transformations of energy in the active muscle, namely (1) the non-oxidative contractile process and (2) the oxidative recovery process.

The effects of temperature have been investigated, and it is probable that the method provides the only satisfactory means hitherto invented of carrying out this important investigation. In single twitches increase of temperature, from 0° to 25° C., causes a decrease of the initial heat-production. The tension developed in an isometric twitch also is slightly decreased from 0° to 20°, though in a smaller degree. The "efficiency" of the muscle in developing tension therefore rises as the temperature rises, and at the higher temperatures the rise may be quite obvious. The heat-production in a short tetanus (0·5 sec.) on the other hand seems almost to be unaffected by temperature. The effect of alcohol is, in lower concentrations (2 to 3·5 %),—after a short increase—a proportional decrease of heat and tension. At higher, but still reversible, concentrations the development of mechanical energy is extinguished while one third and more of the initial heat is still produced. It is therefore possible to separate two parts in the contractile mechanism, *sensu strictiori*, namely one part which provides free energy, and another which transforms this energy into mechanical potential energy or work. It is possible not only to influence this latter contractile portion of the mechanism, but also the energy-providing part, viz. by the use of hypotonic Ringer's solution. In this case we find a proportional decrease of heat and tension, both being extinguished (reversibly) at the same time. The same is true for solutions containing an excess of potassium, except that the change produced is irreversible. It is possible therefore to act upon at least three different parts of the chain of processes in active muscle (1) upon the oxidations (cyanide), (2) upon the initial liberation of energy (temperature, hypotonic solutions etc.), and (3) upon the transformation of this energy into the mechanical response (alcohol, temperature). It is possible that a fourth point in the mechanism can be isolated, viz. the transformation of energy derived from oxidative breakdowns into the restored potential energy of the "recovered" muscle. I have found previously that temperature has an effect on the oxygen used by the heart muscle, directly in the opposite direction to its effect upon the heat-production: the oxygen used for a given mechanical response rises, while the heat-production falls, with rise of temperature. If this be so we have reached a new point of attack upon the muscular mechanism, the "efficiency" of the oxidative recovery processes and its temperature coefficient.

Binocular vision. By F. W. EDRIDGE-GREEN.

(From the Institute of Physiology, University College, London.)

The perception of binocular relief and solidity is not dependent upon the combination of images falling upon disparate points of the two retinas though when images do fall upon adjacent disparate points they are combined with an alteration in their projection in space. If two photographs taken from the same negative be placed one on either side of the stereoscope a striking appearance of relief is obtained, though the photographs are not stereoscopic photographs and no double images are formed. When these are carefully compared with the same view taken with stereoscopic photographs combined in the usual way, in most cases the perspective is better with the stereoscopic photographs, but in some cases it is difficult to detect any difference. If an ordinary stereoscopic photograph of a scene be placed in the stereoscope and viewed in the ordinary way the usual striking appearance of relief is obtained. If now whilst the eyes are still looking at the photograph, a sheet of white cardboard be dropped over one of the photographs so as to entirely occlude this photograph and only present a white surface to one eye, a striking appearance of relief remains. This fact may be emphasised even more by altering the experiment in the following way. If the cardboard be placed so that only half of one photograph be occluded a careful comparison can be made between the upper and lower halves of the combined visual field. It will be noticed that when there is one picture in the stereoscope and a white blank on the other side, the appearance of solidity is greatly diminished when the eye receiving the impression of the white surface is closed.

We can also obtain pictures in strong relief by combining two halves of a stereoscopic photograph, that is to say, half of the photograph which is usually presented to the right eye is shown on the right side and half of the left is shown on the left side. This may be done by placing a sheet of white paper of appropriate size in the centre of the field of vision; in this way there is no common object in the field of vision and yet the stereoscopic photographs combine into an object in strong relief. It will be noticed that the appearance of relief is in some cases even more marked in this way than with ordinary stereoscopic photographs.

It would appear that in ordinary binocular vision the right eye dominates for the right side and the left eye for the left of the combined field unless the position of two points in space be noted when one eye, usually the right, dominates. The mind projects outwards the appearance of a solid object which would give rise to the images falling upon the retinas. This also applies to those cases in which images falling upon disparate points are combined, as for instance when the images of two daggers are combined in the stereoscope and appear as a dagger standing straight out from the table; the images in the stereoscope are similar to those thrown on the two retinas by a dagger standing straight out from the table. When the mind is not able to project the image of an object in space which would produce the two images, double images or binocular struggle or rivalry take place. Binocular struggle is absent when the half photographs are combined in the above way.

Some points in the action of adrenin. By W. Burridge. (*Preliminary communication.*)

Adrenin has a twofold action on the perfused heart of the frog, a primary depressing action and a secondary favouring action.

Whether the primary depressing action be observed or not depends on the effective concentration of the calcium salts in the perfusing solution. If the perfusing solution be such that a reduction of its calcium content to one-fifth the original amount, say, does not result in any material reduction in the amplitude of the spontaneous contractions, then a very large concentration of adrenin must be added to the original solution to depress the amplitude of the spontaneous contractions on perfusion.

But if the heart be perfused with a solution in which the calcium salts are barely adequate, or slightly inadequate, for the maintenance of the spontaneous contractions at their maximum amplitude, relatively small concentrations of adrenin have a visible primary depressing action. By increasing the strength of adrenin the heart can be stopped in the dilated and electrically inexcitable condition. In general terms it may be stated that the more feebly the heart beats at the time of making the experiment, the less is the concentration of adrenin required to stop it in the dilated condition. This action of adrenin is antagonised by calcium salts.

But on washing away the adrenin, the heart not only restarts beating but is capable of maintaining good spontaneous contractions on a solution of much lower calcium content than was the case before the adrenin was perfused. If the strength of adrenin be not sufficient to stop the heart in the dilated condition augmentation follows the primary depression.

In every case examined it was found that when the augmenting and accelerating action of adrenin was present the heart was capable of maintaining activity on a solution of much lower calcium content than was the case before treatment with adrenin. This condition of increased sensitiveness to calcium persisted for some time after removal of the adrenin from the perfusing solution.

The favouring action of adrenin might apparently be as great when the strength employed was 1 in 10,000,000 as it was when the strength was 1 in 250,000 but whereas the latter induced also marked primary depression of the heart, the former was only observed to augment.

If the heart were 'inhibited' by adding potassium salts to the perfusing solution, the addition, now, of adrenin to this solution was followed by resumption of spontaneous beating.

Assuming this favouring action of adrenin to reproduce sympathetic nerve stimulation, it would follow that as a result of sympathetic stimulation the heart has an increased responsiveness to the calcium salts in the solution perfusing it. The persistence of this changed state long after removal of the adrenin from the perfusing solution (15 m.) indicates that a changed state of aggregation of certain heart colloids is an important factor in this process, rather than the local release of calcium salts suggested by Howell and Duke[1].

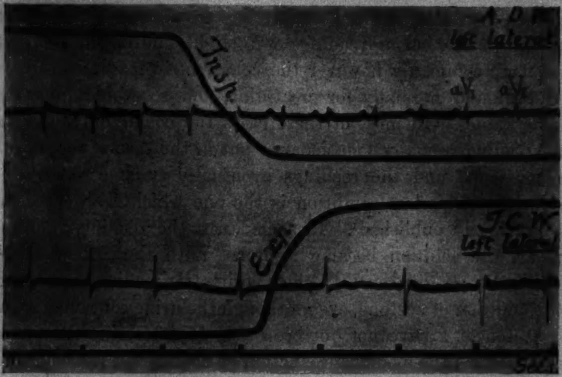
Voluntary reversal of the human electrocardiogram by deep respiration. By A. D. WALLER, M.D., F.R.S.

At a previous meeting of the Society I summarised the effects of the respiratory movements upon the amplitude of the ventricular spike V_I by the general statement that as a rule with inspiratory descent of the diaphragm the electrical effect is weakened in the strong leads (left superior and right inferior), strengthened in the weak leads (right superior and left inferior). (This rule does not however apply to all sorts of hearts, *e.g.* in the horizontal heart the right inferior peak V_I is increased by inspiration.)

The effect of respiration is greater in the cases of the weak than in those of the strong leads.

[1] This *Journal*, xxxv. p. 131. 1906-7.

As regards the two weak leads the effect of respiration is greater upon the left inferior than upon the right superior, so great indeed that it is possible for a person with a *negative* left inferior V_I (horizontal heart) to render it positive by maximal descent of the diaphragm, and for a person with a *positive* left inferior V_I (oblique heart) to render his V_I negative by maximal ascent of the diaphragm.



Upper line. Left lateral record of A. D. W.; the normally negative spike is rendered positive by extreme inspiration.

Lower line. Left lateral record of J. C. W.; the normally positive spike is rendered negative by extreme expiration.

This has been done in the two cases of A. D. W. and J. C. W. respectively. The experiment is one that can be repeated by anyone having the suitable heart, and serves when understood as a caution against admitting too hastily a negative R_{III} as a sign of left ventricular hypertrophy or of interruption of the right branch of His's bundle.

The effect of acetone and aceto-acetic acid on the estimation of creatinine and creatine. By R. A. Krause.

Different results have been obtained by different workers, including myself, in trying to determine the manner in which acetone and aceto-acetic acid affect the creatinine and creatine determinations, and this applies especially to acetone. I have therefore again examined the effect of adding these substances to a number of normal urines, obtained from different persons and at different times. So far, my results show that even when using the same sample of acetone, no matter in what percentage, the effect on the colorimetric readings for creatinine is not always the same. It must be remembered that a deepening of the colour reaction tends to mask the presence of creatine, whereas a lightening of the colour reaction would increase the values for creatine and might therefore give results indicating the presence of creatine in a urine which in reality does not contain any creatine at all. With some normal urines the colour readings were lighter after the addition of acetone, more commonly however the colorimetric readings were lower than those found normally for creatinine, and so would give higher creatinine values. In most of these cases the colour did not fade, so that the readings remained stationary; but in the case of some of them the colour faded and the readings eventually became those of the normal urine. This last condition is the one which I observed in my results previously published[1]. In some cases the readings went even higher, *i.e.* the solution became lighter. With the urines of three normal persons no difference in the colorimetric readings was obtained after the addition of acetone, even although the urine contained as much as 2 °/₀ acetone. I have not yet been able to determine the factor in the urine which thus modifies the action of acetone on the colorimetric readings for creatinine.

After the addition of aceto-acetic acid to the urine the colorimetric readings were always found to be higher, as **Graham** and **Poulton** state. Whether its effect on the colour reaction used for the creatinine estimation shows variations similar to those observed with acetone I have not been able to determine, as I have not examined a sufficient variety of urines. It was found, however, that sometimes the presence of acetone may completely neutralise the effect of aceto-acetic acid. My previously published findings with aceto-acetic acid may have been due to the presence of some disturbing factor of this kind.

My observations therefore, while not entirely agreeing with those of **Graham** and **Poulton**[2], show nevertheless that the presence of acetone and aceto-acetic acid may introduce a fallacy in estimating creatine and creatinine. In my previous observations in which the excretion of creatine in diabetes mellitus and phlorhizin diabetes was demonstrated, it was pointed out specially that the creatinuria may appear in the absence of an acidosis and that, in fact, of the cases of diabetes mellitus examined none showed an acidosis during the period of investigation. There is therefore no justification for the suggestion that the creatinuria in those conditions is only an apparent one.

---

[1] **Krause.** *Quart. Journ. Exp. Physiol.* III. p. 289. 1910.

[2] **Graham and Poulton.** *Proc. Roy. Soc.* B, LXXXVII. p. 205. 1914.

It goes without saying that the fallacy introduced by the presence of the acetone bodies does not enter at all into the consideration of the excretion of creatine by normal women and normal children[1]. In starvation and in carbohydrate starvation the conditions are more complicated, because in those conditions the onset of creatinuria is practically coincident with the appearance of the acetone bodies. Whether in these conditions the creatinuria observed by Cathcart is an apparent one, as Graham and Poulton state, or a real one, as Cathcart maintains, is at present being investigated.

The excretion of creatine in severe and mild cases of diabetes mellitus. By R. A. KRAUSE.

The excretion of creatine in diabetes mellitus, which was observed by Krause and Cramer in 1910[2] and which has since been confirmed by a number of workers, takes place, as was pointed out at the time, even in mild cases of the disease, in which there is no acidosis. Since the observations of Graham and Poulton[3] have made it possible to exclude the influence of aceto-acetic acid upon the estimation of creatinine and creatine it was of interest to study the degree of error introduced by the presence of acetone bodies in diabetes mellitus. I have recently examined three cases of diabetes: one a severe case with a definite acidosis, secondly a milder case with only traces of aceto-acetic acid and acetone and thirdly a case without any acidosis at all. In the first two cases the urine was examined both before and after the removal of the aceto-acetic acid according to the method of Graham and Poulton. In all three cases creatine was present. The following Table gives typical results obtained from each case.

It is noteworthy that in the mild acidosis case, which gave a negative ferric chloride test and a faintly positive nitroprusside (Rothera's) reaction, the differences in the readings for creatinine obtained before and after the removal of aceto-acetic acid were so small as to fall within the limit of error, in other words the amount of acetone bodies

---

[1] Krause. *Quart. Journ. Exp. Physiol.* IV. p. 293. 1911. *Ibid.* VII. p. 87. 1913.

[2] Krause and Cramer. *Proc. Physiol. Soc.* July 1910, *Journ. of Physiol.* XL. Krause. *Quart. Journ. Exp. Physiol.* III. p. 289. 1910.

[3] Graham and Poulton. *Proc. Roy. Soc.* B, LXXXVII. p. 205. 1914.

Degree of acidosis	Preformed creatinine nitrogen plus aceto-acetic acid	Preformed creatinine nitrogen minus aceto-acetic acid	Total creatinine nitrogen	Creatine nitrogen
None	—	0·273	0·294	0·021
	—	0·273	0·303	0·030
Mild	0·386	0·386	0·477	0·091
	0·198	0·195	0·280	0·085
Severe	0·186	0·191	0·319	0·128
	0·209	0·248	0·301	0·053
	0·250	0·307	0·415	0·108

present did not appreciably affect the creatinine and creatine estimations.

This raises the question at what degree of acidosis the fallacy introduced by the acetone bodies becomes negligible. By carrying out observations with normal urines to which varying quantities of aceto-acetic acid had been added, it was found that this acid will not appreciably interfere with the creatinine and creatine determinations as long as the ferric chloride reaction is negative or even faintly positive. Incidentally it may be mentioned that the nitro-prusside test, as modified by Rothera, is much more sensitive for aceto-acetic acid than for acetone, as was pointed out by Hurtley[1].

The "dead space" in breathing. By J. LINDHARD, *Finsen-Institute, Copenhagen.*

Recently Krogh and I[2] published a method of determining the "dead space" in breathing, which is sufficient for most purposes. As the method is rather difficult to manage and requires a special apparatus it would be of great interest to find out some scale, according to which it might be possible to estimate approximately the volume of the "dead space." Usually an approximation will be quite sufficient, but sometimes, *e.g.* when calculating the alveolar-tensions from the composition of the expired air in persons with a very shallow respiration (tidal air 300 c.c. or less), even the most careful determination, according to any method hitherto published, is not accurate enough.

In order to find a suitable basis for such an estimate I have determined the "dead space" on a number of subjects, men as well as women. I have then attempted to compare the results with different

[1] Hurtley. *Lancet*, CLXXXIV. p. 1160. 1913.

[2] *Journ. of Phys.* XLVII. p. 30. 1913.

body measurements (total length, weight, volume of lungs etc.) and found that the best index so far obtainable was the length of the trunk measured in the erect sitting position of the subject from the seat to the angle between the chin and the neck (*os hyoideum*). For using this I find the following formula for estimating the "dead space":

	Women	Men
Length of the trunk	$66 \pm n$ cm.	$68 \pm n$ cm.
"Dead space" at 37° (moist)	$115 \pm 7n$ c.c.	$140 \pm 7n$ c.c.

In all female subjects except Miss R. the agreement between the observed and the calculated values is as good as might be expected. For her I have four determinations, the first two give 75 c.c., last two 98 c.c. for the "dead space." I have strong reasons to believe that the first figure is right; unfortunately the experiments with Miss R. could not be continued.

In the male subjects the difference between the calculated and observed values is wider, and these differences are certainly real, the subjects concerned being well experienced. Only in the case of V. M., however, the use of the calculated "dead space" instead of the determined might occasionally give rise to more serious errors.

Krogh and I have previously stated, that the "dead space" is essentially the same at rest and during muscular work, and that

Table of experiments.

Subject	Length of trunk (cms.)	"Dead space" observed		Calculated from formul.	Deviation of obs. value
		at ca. 20°	at 37°		
Miss A.	66	(106)*, 105, 104, 99, 106 average 104	115	115	0
Mrs M.	65	99, 90·5, 94·5, (98), 93 average 95	105	108	−3
Miss R.	61	78·5, 72, 100, 96·5 average 87	96	80	+16
Mrs H.	60	77·5, 67, 78, 65 average 72	79	73	+6
Miss M.	59	56, 48, 71, 62, 66 average 61	67	66	+1
A. K.†	69·5	148	159	150	+9
K. A. H.	69·5	140, 141, 140·5, 118·5, 143, 163 average 141	155	150	+5
J. J.	69	118·5, 99, 119·5, 106, 112·5, 99, 106, 125 average 111	122	147	−25
V. M.	68	106, 95, 91, 94, 86 average 94	104	140	−36
J. L.†	63·5	98	109	108	+1

* Brackets indicate a small irregularity in the experiment concerned, which has been considered insignificant for the result.

† The figures for A. K. and J. L. have been taken from the previous paper.

probably the upper or upmost part of the air-passages, especially the nose, constitutes the primary resistance when the ventilation is strongly augmented. In a respiration experiment, the subject breathing through a mouthpiece and having his nose closed by a clip, the air current goes through the mouth and when the ventilation is increased the mouth cannot be kept closed. The cavity of the mouth is much more variable in volume than that of the nose owing to the movable and elastic *diaphragma oris*; it is possible to hold more than 100 c.c. of water in the mouth with closed lips and without filling up the *sulci alveolo-buccales*. Alterations in volume of the mouth cavity will alter the "dead space." I have demonstrated this fact in a few double determinations, the one undertaken with "normal" mouth, the other with large mouth.

	Normal mouth	Large mouth
J. L. "Dead space" at 20°	114	134
	112	134·5
	108	137

I have further observed, that in respiration experiments during muscular work I always open the mouth to a certain degree. To see how far this habitual position of the mouth affects the "dead space" I have made a series of determinations with the following result:

J. L. "Dead space" at 20° C. in c.c., 123, 124, 120, 120, 128, 112, 116·5, 119, 129, 109. Average, 120. At 37° C., 132.

The experiments show that the "dead space" is increased by 23 c.c. However, especially in work experiments such an increase is for most purposes quite irrelevant.

Note on the relation between the electrolyte concentration of some neutral perfusion liquids and the frequency of beat of the frog's heart. By MARY D. WALLER, B.Sc.

The object of the following series of observations was to determine the relation between the concentration of physiologically effective perfusion fluids and the activity of the heart as measured by the frequency of the beat.

The observations were made upon the frog's heart (*Rana temporaria*) prepared as described by Mr Mines[1], at whose suggestion they were undertaken.

By preliminary experiments it was ascertained that under constant conditions of temperature and pressure the frequency of beat rises and falls with rising and falling concentration of the Ringer solution in a remarkably regular way for long periods.

---

[1] Mines. *Journal of Physiology*, XLVI. p. 190. 1911.

Thus *e.g.* in one experiment (see Fig. 1) the normal frequency with the original solution

at concentration = 100 is 36 per minute
„ „ = 75 „ 32 „ „
„ „ = 50 „ 28 „ „
„ „ = 40 „ 24 „ „
„ „ = 30 „ 20 „ „

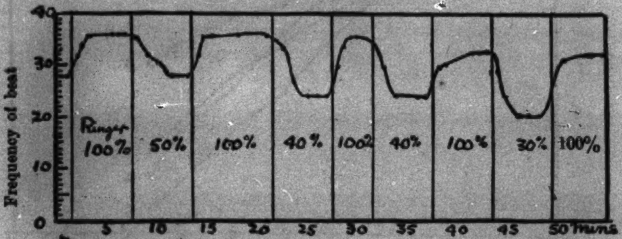


Fig. 1.

The procedure was as follows:—

A normal perfusion liquid (Ringer KCl M/10 2 c.c., $CaCl_2$ M/10 2 c.c., $NaHCO_3$ M/8 1 c.c., NaCl M/8 to 100 c.c.) was prepared; its concentration was taken = 100. This normal solution was diluted by the addition of certain proportions of an isotonic (but physiologically ineffective) solution in which a non-electrolyte, *i.e.* cane sugar or urea, took the place of NaCl, the other constituents remaining as before.

The effects of the more or less concentrated solutions were compared with that of the original solution by observing the beat frequencies during perfusion by the respective liquids.

The frequency of beat was counted for half-minute intervals during periods of perfusion of five to six minutes—a time that was found to be sufficient to allow the frequency to settle to its steady value when the perfusion liquid was changed.

In considering the relation between alterations of frequency caused by alterations of concentration of the Ringer solution, it is convenient to express the fall as a fraction in which the original frequency is the denominator and the altered frequency the numerator or f/F. Thus in Fig. 1 where the original frequency is 36 and the altered frequency 28, this ratio or quotient is $\frac{28}{36}$ or $\frac{78}{100}$ when the concentration is 50 %.

The relation between concentration and quotient for experiments with Ringer and cane sugar is given in Fig. 2, to show how the frequency of beat diminishes with diminution of NaCl in the perfusion liquid.

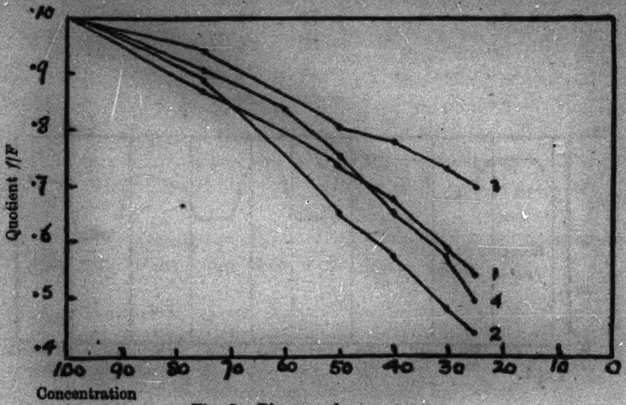


Fig. 2. Ringer and cane sugar.

(Similar curves were obtained by using a Ringer solution in which the Cl ion was replaced by Br, I and NO_3, diluting the Ringer as before by different proportions of the cane sugar and urea solutions.)

Excitation of the sudo-motor nerves of the cat's foot by condenser discharges. By W. W. Waller, M.B.

The sudo-motor electrical response was first demonstrated by Luchsinger and Hermann[1] on the pad of a curarised cat under artificial respiration, and further studied by Waller[2] on a decapitated cat *post mortem*. The object in both cases was to observe only the sudo-motor electrical response of the cat pad, uncomplicated by the electrical effect of muscular contraction and of shifting contact. By reason of the great length of latency of the sudo-motor response it is however possible by means of a string-galvanometer to observe and record the successive effects of both muscular and glandular response when both are present; the indication of muscular response serving as a time-signal to the subsequent glandular response.

---

[1] Hermann and Luchsinger. *Pflüger's Arch.* xvii. p. 310. 1878.

[2] A. D. Waller. *Proc. Roy. Soc.* lxv. p. 207. 1899. *Ibid.* lxxiii. p. 92. 1904.

Stimulation by a single condenser discharge was applied by platinum electrodes to the sciatic nerve of a cat killed under chloroform by puncture of the medulla, the circulation being maintained by artificial respiration. The large pads of the two hind feet were led off to the galvanometer by unpolarisable electrodes. Excitation by a condenser was used in order to learn what energy at what rate was required for the muscular and glandular responses respectively. The resistance in the exciting circuit was measured. With a small stimulus (*e.g.* less than 1 erg) only the muscles contract; there is no electrical response of the pad. With a large stimulus (*e.g.* above 1000 ergs) the muscles of the leg are seen to twitch immediately, and about two seconds later the galvanometer is deflected in a direction indicating an ingoing current of the pad on the stimulated side. As can be seen on the record the two phenomena are separated from each other by an interval of time which is long enough to be measurable by stop-watch.

Electrical response of a cat's pad to a single condenser discharge (2*mF*, 20 volts, viz. 4000 ergs) applied to the sciatic nerve. The small waves at the beginning of the record indicate the muscular twitch of the limb; the subsequent long wave indicates the electrical response of the sweat glands. The E.M.F. of this response is approximately $\frac{1}{100}$ volt and its direction through the skin is from without inwards. Its latent period is 1·7 seconds.

It has always been obvious in repeating this experiment that whereas a small energy discharge with a sharp gradient is sufficient to excite musculo-motor fibres in the sciatic nerve, the excitation of its sudo-motor fibres requires a relatively large energy discharge of more gradual gradient.

Further experiments—not yet completed—have been undertaken to determine the numerical relation between the optimal minimal stimulus in the two cases; the following numbers will serve to indicate the order of difference under ordinary conditions, *i.e.* without regard paid to temperature or moisture or state of nerve:

$R = 10{,}000$ ohms	F capacity	V voltage	$5FV^2$ energy	$V/FR \times 10^6$ gradient	$T_{\frac{1}{2}}$, time of fall of energy to $\frac{1}{2}$
Muscle-nerves	0·02	1	0·1 erg	5000	0·000139 sec.
Sweat-nerves	1·00	10	500 ergs	1000	0·006930 sec.

By the kindness of Prof. Lapique I was able under his control and guidance to obtain some preliminary time-values for the two kinds of nerve-fibres, for excitation by the constant current and by condenser discharges. The characteristic time of excitation ("chronaxie") came out by both methods as approximately 0·003 sec. for the sudo-motor response as compared with 0·0003 sec. which is the chronaxial value of musculo-motor nerves as determined by Prof. Lapique. These figures indicate that the response to excitation of musculo-motor fibres in the sciatic nerve is about ten times as rapid as that of sudo-motor fibres.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

June 27, 1914.

The infra-red absorption bands of hæmoglobin. By H. HARTRIDGE and A. V. HILL.

Haldane and Lorrain Smith[1] found that in sunlight "the affinity of hæmoglobin for CO must have been diminished to at least $\frac{1}{20}$." Presumably this refers to a diminution (to $\frac{1}{20}$) of the equilibrium constant K of the reaction,

$$HbO_2 + CO \rightarrow HbCO + O_2.$$

The maximum work[2] that a reaction can perform, per grm. molecule transformed, is well known to be $RT \log_e K$, so that in sunlight the maximum work of the above reaction is reduced from $RT \log K$ to $RT \log K/20$ calories, *i.e.* by $RT \log 20 = 1850$ calories. Hartridge[3] found the same: in one experiment in the dark there were only 4 % HbO_2 and 96 % HbCO, while in the light there were 60 % HbO_2 and only 40 % HbCO. The equilibrium constant K had therefore changed in the light to $K/36$, and the "free energy" of the reaction had diminished by $RT \log 36 = 2240$ calories, which is in fair agreement with Haldane's figure. In this reaction therefore one is enabled to picture a *light engine*, which by a cyclic process can seize on radiant energy and trans-form it into work. If the reaction be allowed to go → in the dark yielding work $RT \log K$, and be then reversed in the light absorbing work $RT \log K/36$, we obtain on the whole a balance of 2240 calories of work each time the process is completed: *we have a reversible light engine storing 2240 calories of work in each cycle, at the expense only of the incident radiant energy.*

---

[1] *Journ. of Physiol.* xx. p. 504. 1896.

[2] The "free energy." R is the gas constant $= 1{\cdot}98$ calories, and T the absolute temperature.

[3] *Journ. of Physiol.* XLIV. p. 21. 1912.

Now this quantity of energy is large, compared with that provided by all other known light reactions (excepting chlorophyll): it seemed possible therefore that there might be differences between the light-absorption of O_2Hb and COHb just in that spectral region where the energy of the radiation is greatest, viz. in the red and infra-red. This has proved indeed to be the case. The violet waves provide extremely little energy, compared with the longer heat-waves, and in most chemical reactions play only the part of a katalyst. In connexion with the "Glassworkers' Cataract" Committee of the Royal Society we have had made a highly sensitive spectrophotometer for investigating infra-red absorption, and incidentally we have used this apparatus for investigating the infra-red absorption by solutions of reduced, oxy-, and carboxy-hæmoglobin. The following are some typical experiments.

Exp. i. COHb and O_2Hb: 3·2 %. Absorption reckoned in % per mm. traversed. Wave length λ in millionths of a millimetre.

Wave-length		680	700	750	800	850	900	950
Absorption	COHb	14·1	12	11·2	9·8	9·6	9·5	6·9
"	O_2Hb	12·9	12·6	14·5	15·6	19	19·5	17·2

Wave length		1000	1050	1075	1150	1250	1350
Absorption	COHb	6·6	5	3·2	4·3	3·2	2·8
"	O_2Hb	16·6	11	7·6	5·4	3·3	2·4

The visible spectrum ends at 810 $\mu\mu$. The figures are corrected for the absorption by the water. It is seen that over a wide range of λ the absorption of O_2Hb is double that of COHb.

Exp. ii. Reduced Hb and O_2Hb. 7·5 %. As before.

Wave length		600	635	670	700	750	800	850
Absorption	COHb	64	31·5	22·3	22·1	20	18·7	16·5
"	O_2Hb	51	29·8	24·3	24·8	25·6	27	29·2
"	reduced Hb	*	*	*	*	47·5	42·5	38

Wave length		900	950	1000	1080	1180	1280	1350
Absorption	COHb	12·9	14·3	13·3	11·9	8·7	7·2	5·5
"	O_2Hb	30·5	28·6	25·3	19	—	—	—
"	reduced Hb	35·1	30·1	25·3	21·5	17·2	15·2	11

* Too great to read.

It is seen therefore that the absorption by reduced Hb is greatest, the absorption by O_2Hb less, and that by COHb far smaller still. This difference in the absorption by COHb and O_2Hb would obviously be sufficient to account for the large energy change involved in the action of light on the equilibrium: it remains however to determine whether it really is the infra-red rays which affect the equilibrium, and this investigation we hope to carry out shortly. In the meantime one important practical application of the results described can already be foreshadowed. The differences in red and infra-red absorption of Hb, O_2Hb and COHb *may obviously be used as a means of determining the amount of each compound in a given solution*: and the absorption of COHb once exactly known can be used as a means of determining the *total hæmoglobin content of a solution.* It will probably be possible to use the total radiation between (say) 700 $\mu\mu$ and 1300 $\mu\mu$, and not only one particular wave length, for the purpose. In that case a simple blackened thermopile to receive the radiation, a Nernst filament and a moderately sensitive galvanometer should be sufficient to enable one to determine with some degree of accuracy the amount of O_2, CO, and reduced hæmoglobin present at one time in any given solution. The determinations should be easily and quickly carried out, and once calibrated the apparatus should never need any further alteration. With Mr G. Winfield we hope shortly to be able to describe more fully the possibilities and difficulties of the method.

Creatinine and creatine in starvation. By G. GRAHAM and E. P. POULTON.

Cathcart and Orr (1), (2) have recently referred to a paper published by us (3) in which we confirmed Greenwald's (4) statement that the estimation of creatinine was rendered inaccurate by the presence of aceto-acetic acid and in which we had further shown that the error caused by the aceto-acetic acid was roughly proportional to its concentration and that it tended to the belief that creatine was present. We also maintained that "in the many conditions in which the acetone bodies were excreted in the urine the estimations of creatinine and creatine must be inaccurate unless the precaution was taken of removing the aceto-acetic acid from the urine." We also performed three experiments on the effects of carbohydrate starvation in adults under conditions very similar to those under which Cathcart (5) had previously worked and we showed that no "true" creatine was excreted during the three days which the experiment lasted although much apparent creatine was present.

Cathcart and Orr in their last communication stated that complete starvation caused an excretion of "true" creatine amounting to 0·3 gm. on the first day and 0·11 and 0·12 gm. on the 2nd and 3rd days.

We have now performed two complete starvation experiments. In the case of E. P. P., no creatine was excreted until between 58 and 80 hours after the last meal, and then 0·05 gm. was obtained in 22 hours. In the case of G. G., the creatine appeared between 48 and 72 hours, and was 0·06 gm. in 24 hours. The ingestion of ¾ pint of cream (containing about 40 °/₀ fat) prevented any further excretion of creatine during the next two days.

Starvation (*Water ad lib.*).

Subject	Time since last meal, hrs.	Apparent creatinine, grms.	True creatinine, grms.	Apparent creatine, grms.	True creatine, grms.
E. P. P.	10–34	1·75	1·79	0·04	0
	34–58	1·39	1·66	0·27	0
	58–80	1·35	1·62	0·27	0·05
G. G.	24–48	1·34	1·54	0·20	0
	48–72	1·25	1·38	0·13	0·06
	72–96*	1·28	1·38	0·10	0
	96–120*	1·15	1·53	0·38	0

* ¾ pint cream eaten on this day.

On the two occasions on which we found creatine in the urine the difference in the scale reading was very small, only 7·0 to 7·3 (E. P. P.), but it was quite definitely obtained on repeating the observations twice, so that mere experimental error was excluded. In neither case was **Walpole's** diacetyl test for creatine positive, but on adding the same amount of creatine to normal urine, it was not possible to say for certain that any was present by this test.

These results do suggest that traces of "true" creatine are excreted during absolute starvation lasting for three days. But we think that it is rather dangerous to draw too definite conclusions from the very small differences in the scale readings which we obtained, especially as the method is an indirect one and has been shown to be liable to various sources of error. In our experiments the excretion did not begin until from 48 to 58 hours after the last meal, and was smaller in amount than in **Cathcart's** case. In any case these amounts of "true" creatine are small compared with the figures previously reported by various authors.

REFERENCES.

(1) **Cathcart and Orr.** Proc. Physiol. Soc. XLVIII. p. xxi. 1914.
(2) **Cathcart and Orr.** Journ. Physiol. XLVIII. p. 113. 1914.
(3) **Graham and Poulton.** Proc. Roy. Soc. LXXXVII. B, p. 205. 1914.
(4) **Greenwald.** Journ. Biol. Chem. XIV. p. 87. 1913.
(5) **Cathcart.** Journ. Physiol. XXXIX. p. 311. 1909.

The removal of aceto-acetic acid from urine in creatinine and creatine determinations. By G. Graham and E. P. Poulton.

It was stated by Cathcart and Orr[1] that the distillation method, which we used for removing aceto-acetic acid before estimating creatinine in urine, was liable to error, as some destruction of creatinine occurred. If, as Cathcart suggests, creatinine is broken down the result would be too low and this would tend to the belief that creatine was present, and the same conclusion would be drawn, if all the aceto-acetic acid had not been removed.

We have therefore repeated our controls and have found that, using the same precautions as we have previously described (with the extra one that the temperature must not rise above 65° C. to prevent creatine breakdown), no creatinine is destroyed, even if the distillation is continued for 2½ hours.

Creatinine in urine, scale reading	Creatinine after distillation, scale reading,	Time of distillation, hrs.
6·65	6·65	1
6·7	6·8	1¼
7·1	7·1	2½

We have previously shown that no creatine is broken down by our method, but if amounts very much larger than those usually met with are present, a small breakdown does occur above 65° C. but none at 60° C.

Creatinine in urine, grms.	Creatine added to urine, grms.	Creatine converted into creatinine, grms.	Temperature of distillation	Time of distillation
0·109	0·116	0	60° C.	¾ hr.
0·098	0·116	0·004	65–67° C.	¾
0·1005	0·116	0·0035	65–68° C.	¾

When considerable amounts of aceto-acetic acid are present in the urine as occurs in severe diabetes it is better to adopt a modified form of Greenwald's[2] ether extraction method.

The phosphates and other substances are first removed by the method advised by Hurtley[3] in order to prevent emulsification during the extraction with ether. Between 2 and 4 c.c. of 40% yellow ferric chloride are added to 200 c.c. of urine and the precipitate is then filtered off. The urine is acidified with half its volume of normal hydrochloric acid and extracted with ether for 12 hours or longer at a temperature of 50° C. The bulk of the ether is removed by means of a separating funnel and the remainder is blown off by a current of air. The estimation of the creatinine and creatine is then made in the usual way, allowance being made for the hydrochloric acid which was added. No creatinine or creatine is broken down by this method. The diacetyl test is more easily seen on the extracted than on the unextracted urine.

---

[1] Cathcart and Orr. *Proc. Physiol. Soc.* March, 1914.

[2] Greenwald. *Journ. Biol. Chem.* xiv. p. 87. 1913.

[3] Hurtley. Communicated to Physiol. Soc. 1912.

	Creatinine (scale reading)		Creatinine+creatine (scale reading)	
	before extraction	after extraction	before extraction	after extraction
Urine	9·0	9·0	—	—
Urine	6·1	6·1	—	—
Meat extract	8·9	8·9	6·08	6·07
Diabetic urine	—	—	6·2	6·2

The oxidation of cholesterol. By F. W. LAMB. (*Demonstration.*)

Pure cholesterol heated to melting point with free access of air gives a product which reacts in chloroform solution like oxycholesterol, to Lifschütz acetic sulphuric-acid reaction, and to the methyl sulphate reaction of Rosenheim.

These oxycholesterol reactions can also be obtained by heating in glacial acetic acid or in glycerol with access of air. Mixtures of fatty acids and cholesterol kept at body temperature in air or oxygen quickly give evidence of the oxidation of the terpene, disappearance of the fluid crystalline phase, increase of weight, diminution of pressure in closed vessels, and the appearance of the oxycholesterol reactions both colour and spectral.

For example, the mixture with oleic acid rapidly absorbs oxygen from the first day and in the course of three weeks passes from a semi-solid to a honey-like consistence. When at this stage—there is no double refraction at any temperature from that of room upwards—it tends to form an emulsion in water, and the titration acidity has increased to twice the original amount. The staining reactions show points of interest, nile blue stains the globules a deep blue, some of the smaller however show a green tint, some of the larger a pleochroism. After the action of potassium biochromate in saturated solution, (*a*) at room temperature, the hæmatoxylin staining begins on the 5th day, the osmic acid reaction remains positive for a couple of weeks, (*b*) at 37° C. the hæmatoxylin lasts from the 2nd to the 5th day, the osmic reaction is only present in the large globules on the 5th day.

After osmication the globules of the mixture are insoluble in alcohol, xylol, and acetone, but soluble in ether or chloroform, all tested at room temperature.

The ease of oxidation of cholesterol in the presence of fluid fatty acids is interesting in view of their frequent association in the tissues. Any quantitative estimation of cholesterol should not involve drying of the tissue in air. The properties of the oxidised oleic acid mixture explain some of the difficulties in detecting microchemically the presence of cholesterol after absorption of this body when it has been fed in fatty acid mixture.

A conducting path between the right auricle and the external wall of the right ventricle in the heart of the mammal.

By A. F. STANLEY KENT. (*Demonstration.*)

(*From the Physiological Laboratory, University of Bristol.*)

When in the heart of a mammal one severs all the structures which connect the auricles to the ventricles with the exception of a strip of tissue on the right lateral aspect of the organ, spontaneous beats arising in the auricle still pass through to the ventricle and evoke ventricular beats. And when, after such an operation, the heart has ceased to beat spontaneously, auricular beats excited artificially also pass through to the ventricle and are followed by ventricular beats.

Since under the conditions described the section has passed through the ventricular septum as well as through the whole of the wall of the left ventricle it follows that the transmitted beats must have passed over a conducting path contained in the only part of the auriculo-ventricular connection remaining, viz. in the lateral wall on the right side of the heart.

Histological evidence of the existence of a muscular connection between the auricle and the ventricle in this situation has already been brought forward[1]. It is supported by the facts cited and demonstrated in the present communication.

This research has been assisted by grants from the Government Grant Committee of the Royal Society, from the British Association for the Advancement of Science, and from the Research Fund of the University Colston Society.

---

[1] *Proc. Physiol. Soc.* November. 1892. *Ibid.* March 14th. 1914.

Some actions of magnesium chloride. By W. BURRIDGE. (*Preliminary communication.*)

An inhibitory action of magnesium sulphate on the respiratory centre has been demonstrated by Meltzer and Auer. It is also possible to elicit phenomena of an inhibitory character from the perfused heart at a certain stage in the action of magnesium chloride.

The heart slows and suddenly stops in the dilated condition. Excitability to induced shocks is still preserved but the amount of contraction elicited by each suitably spaced single shock grows gradually less until finally the heart may be no longer visibly responsive to that mode of excitation.

But if a second stimulus be sent in close enough to a former one which barely elicited any response this second stimulus may be followed by a well-marked contraction. And by sending in a series of appropriately timed single shocks staircase phenomena are produced. The staircase can be interrupted at any moment by increasing the interval between two successive stimuli. A fresh staircase starting from zero can then be again produced.

Atropine and adrenin bring the excitability back to normal and the heart restarts beating in presence of the magnesium salts.

Summation of contractions can be obtained from hearts treated with magnesium salts.

Note on the potassium contractions. By W. BURRIDGE.

The present author has already shown that potassium salts can evoke from the heart of the frog two contractions which differ from each other in regard to several particulars. Some further examples are given in the accompanying figures.

The tracings were all obtained from a single heart and are given here on account of the numerical relations which they show to exist between the two contractions.

The two contractions appear in definite order, and the diagrams show that the amplitude of each was a variable quantity. But if we term x the amplitude of the first contraction at the moment the second was superposed on it, and the amplitude of that superposed second contraction y, we find a relationship of the form $x+y=C$, where C is some constant quantity. The actual value of C in these diagrams is 3·5 cms.

The potassium contraction may also be superposed on the contractions induced by barium salts, ether, chloral hydrate etc. with similar results.

Key to Illustrations.

↳ 5 % KCl—Perfusion of 5 % KCl in 0·6 % NaCl begun here.

T.C. = ‘tonic contraction.’

C.E. = ‘contraction effect.’

PR. The pressure was suddenly raised here by pinching the rubber tubing connecting reservoir with cannula. This procedure brings on the contraction effect.

↳ Perfusion of Ringer’s solution begun.

P.V. The KCl solution was ‘pumped’ out of the heart and each pump stroke notches the tracing.

Between the marks + and ϕ the heart was faradised.

In every heart so far examined it has been found possible by using potassium salts to ascertain a certain value C which is a constant quantity for that heart. This value C represents the maximum amplitude attainable by the spontaneous contractions in that heart. These spontaneous contractions are, however, apparently capable of indefinite variation between the limits zero and C. But no matter what may be the amplitude of the spontaneous contractions at any moment, it is usually possible to evoke from the heart at that moment by using potassium salts the constant contractility C.

The value of C seems to be influenced only by those substances which induce rigor. The majority of drugs though greatly influencing the amplitude of the spontaneous contractions do not correspondingly influence the value of C.

C thus appears to measure the maximum contractility of the heart. As a further corollary the contractile substance is single.

Some effects of epinine. By W. BURRIDGE. (*Preliminary communication.*)

Epinine is a substance which can alter the reaction of the heart to the calcium salts in a solution perfusing that organ.

At the time when the substance induces its already ascertained sympathomimetic action it is found that the heart can maintain activity on a solution of much lower calcium content than was the case before the epinine was added to that solution.

If the heart be perfused with a solution of such low calcium content that the beats cannot be recorded in the usual way, the addition of one part of epinine to five millions of the perfusing solution is not only followed by the appearance of good contractions but in addition the heart can maintain activity on that solution over long periods of time.

Epinine can restart beating in hearts inhibited by potassium salts.

Large doses of epinine render the heart less susceptible to certain actions of calcium than was the case before the treatment with the drug.

Adrenin and perfusing solutions. By W. BURRIDGE. (*Preliminary communication.*)

The heart of the frog beating on and circulating its own blood behaves as if perfused with a fluid of very high potassium concentration, and as if the heart wall itself were highly sensitised to calcium.

This behaviour is changed immediately after any of the ordinary inorganic salt perfusing solutions is substituted for blood. The heart now behaves as if perfused with a fluid of very much higher effective calcium concentration than that of blood.

It has not yet been found possible to compound a solution in which the heart behaves as if perfused with blood. But a very close approximation to such behaviour has been obtained by adding sufficient potassium, or nearly so, to stop the heart in the dilated condition, and then restarting by adding a trace of adrenin to the perfusing solution (1 in 10,000,000). Solutions of very low calcium concentration have been used—not greater than 0·002 °/₀ $CaCl_2$. The heart has been found capable of maintaining activity apparently unimpaired over eight hour periods on such solutions.

The perfusion of any of the ordinary inorganic salt perfusing solutions is followed by a lessened sensitiveness of the heart to calcium (cf. Clark). The change is induced primarily by the calcium salts in the perfusing solution. In general terms it may be stated that the greater the amount of calcium in such solutions the more quickly will the sensitiveness of the heart to calcium be decreased by their perfusion.

The addition of adrenin to these solutions enables the heart to maintain its activity on them over much longer periods of time. The time may be quadrupled by such addition.

Equilibrium in salt perfusing solutions. By W. BURRIDGE. (*Preliminary communication.*)

If the frog's heart be perfused with isotonic sodium chloride for ten minutes, say, at the end of that time the effective action of a given concentration of calcium salts in

(*a*) the induction of tonus
(*b*) the maintenance of the spontaneous contractions (excitability)
(*c*) shortening the refractory period

is greatly increased in each case.

A shorter perfusion of stronger concentrations of sodium chloride is equally efficacious.

If physiological amounts of potassium chloride, or at any rate what are usually regarded as such (0·03 °/₀ KCl), be added to isotonic sodium chloride and the mixture perfused, the effective action of calcium is only slightly if at all increased at the end of a similar time.

If a mixture of sodium and calcium salts be perfused the heart ceases activity in a condition unfavourable to the actions of calcium mentioned above. The greater the amount of calcium in the mixture the quicker does activity cease, and the earlier the condition of decreased responsiveness to calcium comes on.

Acidity decreases and alkalinity increases, within limits, the efficiency of the calcium salts in a perfusing solution.

Each of the elements in a perfusing solution is, thus, a factor in determining the working efficiency of its calcium salts in certain directions.

As is well known hearts perfused with the usual inorganic salt solutions usually cease activity in a few hours. They cease activity because of a lessened power of reaction to the calcium salts in the perfusing solution. By the employment of various methods activity can be greatly prolonged on these solutions. The common factor found to these methods is that of increasing the sensitiveness of the heart to calcium salts. And by the use of potassium salts it can be shown that hearts which have ceased spontaneous activity on a particular salt solution retain their contractility unimpaired, *i.e.* the value of '*C*' does not change. (See 'Note on Potassium Contractions' above.)

The mere enumeration of the composition of a perfusing fluid gives no information of this continually changing condition of the heart itself.

The condition of the heart at any moment can be expressed in terms of one variable, calcium. The maximum contractility of the heart is ascertained by the use of potassium salts. A perfusing solution constant in composition except as regards its calcium content is then taken and the amount of calcium necessary to elicit beats of half this maximum ascertained. This calcium concentration is taken to be a measure of the working efficiency of calcium in that heart at that time.

It has not been found possible to add any salt or drug to a solution perfusing the heart without altering the working efficiency of calcium as determined by this method.

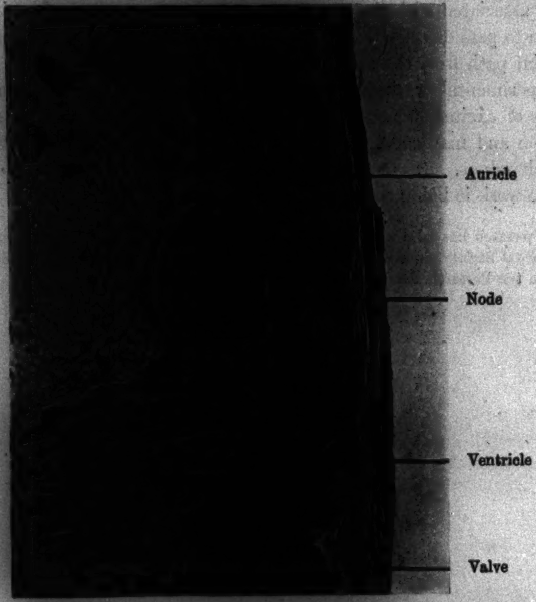
Illustrations of the right lateral auriculo-ventricular junction in the heart. By A. F. Stanley Kent.

(*From the Physiological Laboratory, University of Bristol.*)

An attempt has been made to illustrate the relations of the parts situated at the right lateral auriculo-ventricular junction in the heart of man.

The illustrations consist of untouched photographs and lantern slides exhibited at the meeting together with an untouched photograph reproduced as a figure herewith.

The centre of the figure is seen to be occupied by a rounded mass of tissue representing the right lateral auriculo-ventricular node. This mass is bounded by connective tissue for a part of its circumference, the



Section through the right lateral auriculo-ventricular node in the heart of man. × 30.
(Heart 130327 A. Series A. Section 106.)

connective tissue sheath being discontinuous on the ventricular aspect, and also towards the auricle. The centre of the node is occupied by modified ("nodal") tissue, the details of which may be studied in the photographs taken under a higher magnification and shown herewith.

The vascular supply to the node is abundant.

Portions of tissue forming the peripheral parts of the node resemble auricular muscle in structure.

The node is separated from the adipose tissue of the groove by a definite septum of fibrous connective tissue, whilst towards the cavity of the heart a thick mass of fibrous tissue—which is penetrated to some extent by muscle—separates the node from the endocardium and from the auricular cavity.

The upper part of the node becomes continuous with the muscular tissue forming part of the lateral wall of the right auricle.

Towards the ventricle the muscular tissue of the node is seen to be closely associated with the ventricular muscle. In some sections it may be seen to pass directly from node to ventricle, and to form a continuous muscular path from the auricle to the ventricle.

Experimental work has lent support to the suggestion that the process of excitation may pass across from auricle to ventricle in this situation and may serve to assist in the co-ordination of the action of these chambers.

The work is being continued.

This research has been assisted by grants from the Government Grant Committee of the Royal Society, from the British Association for the Advancement of Science, and from the Research Fund of the University Colston Society.